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THE
SCIENTIFIC PROCEEDINGS
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No. 1.

IRISH PREVIOUSLY SPAWNED SALMON

By

ARTHUR E. J. WENT,

DEPARTMENT OF AGRICULTURE, FISHERIES BRANCH, DUBLIN.

IN recent times there has been considerable discussion, in angling and sporting journals, on the subject of previously spawned salmon. To date no information has been available for any one country as to the proportion of previously spawned fish in the different rivers in any one year, or how these proportions may vary from year to year. In Ireland there are varied views on the value of the "returned kelt" in the stocks of salmon of a river. Some people hold that they are valueless, and others that the larger members of the salmon stocks are previously spawned fish.

The idea that very large salmon must have spawned on at least one occasion is one which "dies hard", despite many investigations in which it has been shown to the contrary. Of course, if one considers the stocks of salmon in a river where the majority of fish are very small, *i.e.*, a large proportion are grilse or peal, then it is true that the heavier members of the stocks will contain a larger proportion of previously spawned fish than the lighter ones. This is shown clearly in the case of the Ballisodare River (Went, 1941, p. 343, Table 9), and, no doubt, applies equally well to other similar rivers. In rivers where the average weight is high the larger members of the stocks are invariably maiden or unspawned fish. For example, during the 1944 season the present author examined the scales of a fair number of large salmon, weight 35 lb or over, from various rivers throughout Éire. Not a single specimen of a previously spawned fish was noted. In all our investigations into Irish salmon, only two previously spawned fish weighing over 35 lb. have been encountered. The first (37.5 lb.) was recorded by Southern (1928, p. 58) from the Shannon, and the second (37.0 lb.) by the present author from the Corrib (1943, p. 284). Taking the recorded data from the Shannon alone, 24 very large spring

fish have been examined having a total weight of 1,011.4 lb. or an average weight of 42.2 lb. Weights up to 56 lb 10 oz have been recorded from the Shannon in these investigations.

In the autumn of 1943 the Fisheries Branch of the Department of Agriculture was approached by persons interested in the River Lee for information on the subject of previously spawned fish in that river. No investigations have been carried

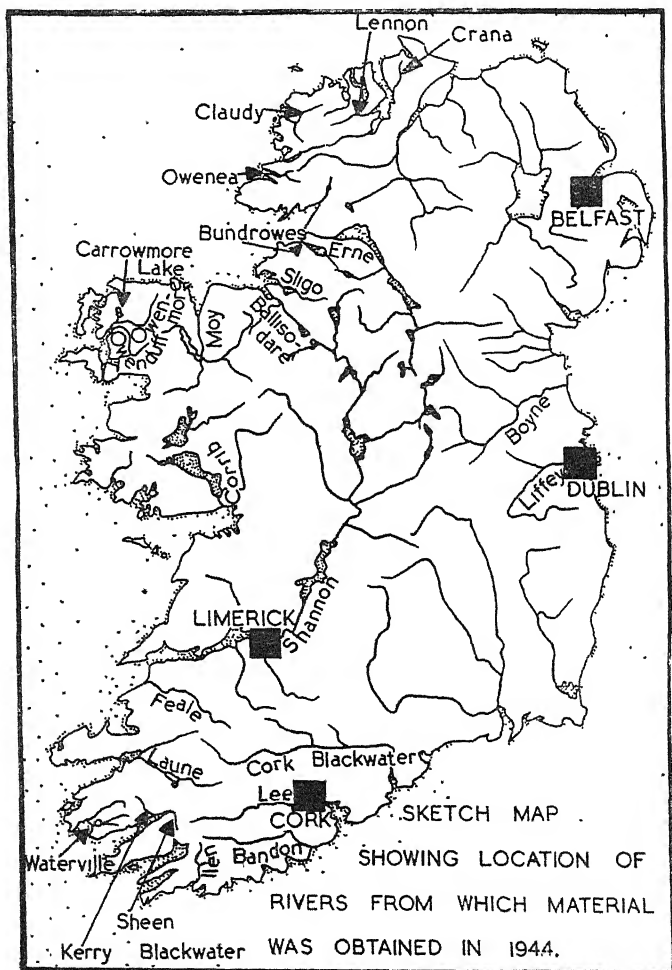


Fig. 1.

out on the River Lee, and it was naturally impossible to give any information on the subject. It was, however, suggested to the persons interested in the matter that if they were willing to collect the necessary material the officers of the Department would work it out. Owing to the stress of other work it was not found possible, at that time, to carry out a full scale investigation into the salmon of the River Lee, but in view of the widespread interest in the subject of previously spawned fish it was decided to extend a modified form of investigation so as to include a number of other rivers in the country. (see map).

Table 1. Showing sources of material and collectors. The rivers are arranged clockwise from Dublin.

| River | Name and Address of Collector | River | Name and Address of Collector |
|----------------------|--|-------------|---|
| Liffey | The late Captain F.H. French Davis, Salmon Pool House, Dublin | Moy | Mr. W.P. Stevens, Moy Fisheries, Ballina, Co. Mayo. |
| Blackwater (Cork) | Mr. G. Fitzgerald, Lismore Estates Co., Lismore, Co. Waterford. | Ballisodare | Mr. F.P. Scott, Two Rapids, Ballisodare, Co. Sligo. |
| Lee | Mr. J. Scanlon, 53 South Mall, Cork | Sligo | Mr. W.F. Middleton, Collooney, Co. Sligo |
| Bandon | Mr. W.J. Seymour, Dunderrow, Kinsale, Co. Cork. | | Mr. J. Jenkins, Sligo. |
| Ilven | Mr. J. Beamish, Skiibereen, Co. Cork. | Bundrowes | Mr. J. Cassidy, Bundrowes, Bundoran, Co. Donegal. |
| Sheen | Mr. W. Johnstone, Estate Office, Kenmare, Co. Kerry. | Erne | Mr. T. MacD. Swan, Erne Fisheries, Ballyshannon, Co. Donegal. |
| Blackwater (Kerry) | Colonel E. Hood, Dromore Castle, Kenmare, Co. Kerry. | Owenea | Mr. F.A. Watson, Riversdale House, Glenties, Co. Donegal. |
| Waterville | Mrs. H. Butler, Waterville House, Waterville, Co. Kerry | Claudy | Mr. J. Hunt, The Hotel, Gweedore, Co. Donegal. |
| Laune | Mr. A. de C. Dodd, K.R.D. Fisheries, Killorglin, Co. Kerry. | Lennon | Sir Harry Stewart, Bart., Fort Stewart, Co. Donegal. |
| Feale | Mr. G. Gleasure, The Square, Listowel, Co. Kerry. | Crana | Mr. I.J. Trew Colquhoun, Buncrana, Co. Donegal. |
| Shannon | Mr. L. Forde, Shannon Fisheries, Limerick. | Boyne | Mr. P.J. Tiernan, Oldbridge, Co. Meath. |
| Corrib | Lt. Colonel E.G.K. Cross, D.S.O., Weir House, Galway. | | Captain R. Law, Rosnaree, Slane, Co. Meath. |
| Owenduff (Ballycroy) | Mr. P.J. Lenehan, Croy Lodge, Ballycroy, Co. Mayo. | | |
| Owenmore | | | |
| Carrowmore Lake | | | |

Table 2. Percentage of previously spawned fish.

| Month | River | | | | | | | | | | | | |
|-------|--------|-------------------|-----|--------|-------|-------|--------------------|------------|-------|-------|---------|--------|----------|
| | Liffey | Blackwater (Cork) | Lee | Bandon | Ilven | Sheen | Blackwater (Kerry) | Waterville | Laune | Feale | Shannon | Corrib | Owenduff |
| Jan. | - | - | - | - | - | - | - | 5.5 | 2.9 | - | - | - | - |
| Feb. | 0.8 | - | 0.2 | - | - | - | - | 7.0 | - | - | - | 23.5 | - |
| March | 0.8 | 2.9 | 1.7 | - | - | - | - | Nil | 2.8 | - | 6.9 | 10.7 | - |
| April | Nil | 1.6 | 1.0 | - | - | - | Nil | Nil | 0.8 | 1.1 | 3.9 | 12.0 | 1.9 |
| May | | 1.4 | 2.1 | - | 1.8 | - | 6.0 | 6.1 | 2.5 | 1.2 | 1.2 | 2.2 | Nil |
| June | | 0.9 | 3.0 | - | 7.2 | - | 5.2 | | 4.3 | 2.2 | 2.1 | 5.9 | 3.8 |
| July | | 5.0 | Nil | - | 12.4 | - | 5.4 | | 5.4 | 6.8 | 10.5 | 19.0 | 11.6 |
| Aug. | | - | Nil | - | - | - | - | - | - | - | - | - | - |
| Total | 0.6 | 2.5 | 1.2 | 8.3 | 8.2 | 3.3 | 5.1 | 5.8 | 3.3 | 1.8 | 4.1 | 7.5 | 4.1 |

| Month | River | | | | | | | | | | | | Total for all rivers examined |
|-------|----------|-----------------|------|-------------|-------|-----------|------|--------|--------|--------|-------|-------|-------------------------------|
| | Owenmore | Carrowmore Lake | Moy | Ballisodare | Sligo | Bundrowes | Erne | Owenea | Claudy | Lennon | Crana | Boyne | |
| Jan. | - | - | - | - | 5.7 | - | - | - | - | - | - | - | 5.5 |
| Feb. | - | - | - | - | Nil | 6.9 | - | - | - | - | - | - | 2.6 |
| March | - | Nil | 17.3 | - | 5.0 | 6.8 | - | - | - | - | - | Nil | 2.6 |
| April | - | 0.6 | 4.8 | - | Nil | 7.2 | 11.6 | - | - | - | - | 0.7 | 3.3 |
| May | - | Nil | 5.0 | 8.2 | Nil | 7.2 | - | - | - | - | - | 1.3 | 3.8 |
| June | - | 5.5 | 1.9 | 8.9 | Nil | 5.2 | - | - | - | - | Nil | 1.0 | 2.3 |
| July | - | 4.6 | 3.2 | 1.9 | 3.3 | 4.0 | 8.8 | - | - | - | 5.7 | 0.8 | 4.4 |
| Aug. | - | - | 6.0 | 0.8 | | 16.9 | | - | - | - | - | 2.2 | 6.7 |
| Total | 10.2 | 1.4 | 4.3 | 2.4 | 4.5 | 7.3 | 9.5 | 2.0 | 1.3 | 7.4 | 5.4 | 1.2 | 4.3 |

Table 3. Comparison of present results with those from previous investigations.

| River | Reference to previous publication | Proportion of previously spawned fish | |
|-------------|-----------------------------------|---------------------------------------|-----------------|
| | | 1944 | Previous result |
| Ballisodare | Went, 1941, p. 342 | 2.4% | 4.8% and 4.7% |
| Owenduff | Went, 1941, p. 178 | 4.1% | 4.9% |
| Erne | Went, 1942, p. 475 | 9.5% | 6.4% |
| Corrib | Went, 1943, p. 295 | 7.5% | 6.0% |
| Waterville | Went & Barker, 1943, p. 100 | 5.8% | 7.2% |
| Shannon | Went, 1943, p. 172 | 4.1% | 5.6% |
| Liffey | Unpublished data | 0.6% | 2.3% |

The material consisted of a single scale, taken from just below the dorsal fin, from as many fish as possible. The scales from the different months were preserved in separate bottles in ordinary water. If the water was changed regularly the scales became free from slime and remained in a satisfactory condition for examination, although, if the water was not changed often, the scales underwent rapid deterioration. In all 28 persons were approached, and of these 25 undertook to collect and, what is more important, did collect material relating to the rivers in which they were interested. In Table 1 a list of rivers investigated has been given together with the names of persons collecting the material. The author wishes to take this opportunity of thanking the collectors for their collaboration in this investigation.

Not all the scales collected in the manner described above are suitable for age determination, as some are replacement scales (*i.e.*, scales which have replaced an original scale lost during some period of life). There is no reason, however, to believe that the elimination of such scales and the investigation of the remaining suitable scales would introduce any error in the results.

In all 25,029 scales were examined, of which 1,078 or 4.3% were from previously spawned fish. Only 12 out of the 1,078 fish bearing spawning marks on their scales, or 1.1% of the previously spawned fish, had spawned previously on two occasions. Not a single specimen of a fish which had spawned three or more times previously was encountered. The proportions of previous spawners in the different rivers have been given in Table 2, only the percentage of the total run being indicated where the number of scales examined did not exceed 300. In the case of the 19 rivers shown below more than the minimum amount of material (300 scales) was available. They have been arranged in order of the percentage of previous spawners.

| | | |
|--------------------------------|------|------|
| (1) Liffey <i>P</i> | ... | 0.6% |
| (2) Boyne <i>P</i> | .. | 1.2% |
| (3) Lee <i>P</i> | | 1.2% |
| (4) Carrowmore Lake <i>L</i> | | 1.8% |
| (5) Feale <i>P</i> | .. | 1.8% |
| (6) Ballisodare <i>L</i> | . . | 2.4% |
| (7) Cork Blackwater <i>P</i> | . | 2.5% |
| (8) Laune <i>P L</i> | | 3.3% |
| (9) Shannon <i>P L</i> | | 4.1% |
| (10) Owenduff <i>P</i> | | 4.1% |
| (11) Moy <i>L</i> | .. | 4.3% |
| (12) Sligo <i>L</i> | | 4.5% |
| (13) Kerry Blackwater <i>L</i> | . | 5.1% |
| (14) Crana | . | 5.4% |
| (15) Waterville <i>L</i> | . | 5.8% |
| (16) Bundrowes <i>L</i> | ... | 7.3% |
| (17) Corrib <i>L</i> | . | 7.5% |
| (18) Ilan <i>P</i> | | 8.2% |
| (19) Erne <i>P L</i> | . | 9.5% |

The Letter *P* adjacent to the name of a river indicates that there is an important public or private fishery in tidal waters which is intensively fished, while the letter *L* indicates that there are one or more lakes on the river system

From the foregoing table it would appear that where there is a large commercial fishery on the tidal waters, there is a tendency for the proportion of previous spawners to be low. On the other hand, where there are one or more lakes on the river the proportion of previous spawners would appear to be high. There are two notable exceptions to this in the Ballisodare River and the River Ilan. The Ballisodare River has on the course of one of its tributaries a fairly large lake (Lough Arrow), but, as will be seen from the map of the watershed of the river given in Went, 1940 (p. 291), salmon do not enter the lake, which may provide an explanation of this apparent discrepancy. In the case of the Ilan no such explanation can be given, but it might be pointed out that the river is a very short one. The Erne is a river which has a large public fishery in its tidal portion, but there is also a lake on the river, the majority of salmon having to pass through this lake before spawning.

It might now be asked how the present results compare with those from previous full scale investigations on Irish rivers. In Table 3 the comparison has been given. With the exception of the Rivers Corrib and Erne the proportion of previously spawned fish was less in 1944 than in the years investigated previously.

As we have a fairly large number of rivers represented in each month of the season we might assume, without being over-speculative, that the monthly combined figures apply in some degree to the country as a whole. In Table 4 the material from each month has been pooled and the proportion of previous spawners calculated.

TABLE 4

| Month | Number of scales examined | | | Percentage of previous spawners |
|----------|---------------------------|--------|--------|---------------------------------|
| | With SMs | Maiden | Total | |
| January | 40 | 693 | 733 | 5.5% |
| February | 38 | 1,398 | 1,436 | 2.6% |
| March | 81 | 2,370 | 2,451 | 3.3% |
| April | 101 | 2,580 | 2,681 | 3.8% |
| May | 77 | 3,223 | 3,300 | 2.3% |
| June | 402 | 8,835 | 9,237 | 4.4% |
| July | 334 | 4,673 | 5,007 | 6.7% |
| August | 5 | 179 | 184 | 2.7% |
| TOTAL | 1,078 | 23,951 | 25,029 | 4.3% |

The estimated proportion of previous spawners for the country as a whole is therefore 4.3%, or, put into another form, the previously spawned fish are equivalent in number to approximately two-thirds of the total catch in Éire by

single rod and line. From this it would appear that the "returned kelts" in most rivers contribute a fairly substantial quota to the stocks of salmon.

That only about one in every twenty fish entering a river is a previous spawner has led to the belief in the great destruction of kelts after spawning. It is unnecessary to cite the many statements throughout the angling literature that 95% or more of fish which have spawned, fail to return to the river for a second time. This misconception can be explained simply in the following way. If we assume that *every salmon survived one spawning*, then with regular runs of salmon of the same strength the proportion of previous spawners would be 50%. In other words a *survival rate of 100%* would, with runs of constant strength, only produce 50% previous spawners. Mr J A Hutton in a recent contribution to the *Fishing Gazette* (No. 3,496, Vol. 126, 22nd April, 1944, pp. 196-197) shows that in the case of the Wye the survival of female kelts was about 20% and those of the males 1.2%, the results for both sexes combined being 10.8%. There is no doubt that male kelts do not survive to make a second return to fresh water as clean fish to the same extent as the females, as the proportion of male previous spawners is usually much less than that of the female previous spawners. Naturally the information for Éire is not as complete as that for the Wye, but in at least two rivers the information available is interesting.

The Waterville (Currane) River is peculiarly circumstanced in that, owing to its narrowness, the fishing weir is carried completely across the river by the authority of an order made by virtue of Section 11 of 26 and 27 Vic. Cap. 114. Instead of the normal two-day weekly close period, however, in this river the weir is open for the free passage of fish for a period of 72 hours. (See Went and Barker, 1943). During the weekly fishing period, therefore, virtually all the salmon entering the river are taken by either the weir or the net operated downstream therefrom. Let it be assumed that 1,000 fish enter Ballinskelligs Bay for the purpose of running ultimately into the Waterville River. Of these fish a quantity, estimated at about 150 in number, will be taken by the open Bay seine nets, leaving 850 to enter the river proper. Of these, four-sevenths on an average will be taken by the weir and net, leaving three-sevenths, or 365 fish, to ascend the river. The *Statistics of Salmon, Sea Trout and Eels* published by the Fisheries Branch of the Department of Agriculture, indicate that from 1927 to 1939 the rod catch was 15.8% of the catch by commercial methods in the Waterville Fishery District in which the Waterville or Currane River is the principal salmon river. Even assuming that only 10% of the net and weir catch, say 64 fish, is taken by the rods then the escapement for spawning will not exceed 301 fish. Assuming that the results obtained in this investigation for the Waterville River apply generally from year to year, each 1,000 fish originally coming in from the sea would contain 58 previous spawners. In other words an escapement of 300 fish, which were allowed to spawn, provides 58 previous spawners in future years or a survival of the spawned fish at a rate of 19.3% for both sexes.

Now let us pass to the Shannon, where accurate records of the fish entering the freshwater portion of the river are available. Shannon salmon are subjected to capture on the estuary of the river by three types of engines, namely (1) Stake Weirs, (2) Drift nets, and (3) Draft nets. After careful consideration of all the

factors involved the author considers that about 25% of the run of salmon native to the Shannon proper above Limerick are taken before reaching Thomond Weir operated by the Electricity Supply Board (Shannon Fisheries). Thus out of every 1,000 fish originally available 750 fish escape the various fishing engines in the Lower Shannon to enter Thomond Weir. This weir is operated without a free gap or passage for fish, by special authority of an Order of the Minister for Agriculture, by virtue of the provisions of the Shannon Fisheries Act of 1938, for what is virtually the whole of the runs of salmon throughout the season (see Went, 1943). The proportion of salmon which the Shannon Fisheries are now authorised to take is 28% of the total run, or 210 fish from our original 1,000 fish, leaving 540 fish to pass upstream. In the Shannon proper the proportion of rod-caught fish to those taken by commercial methods would probably not exceed 10%, but, allowing a total of 20% for all exigencies, about 440 fish would be left to propagate the species. From Table 2, however, it is clear that 41 out of every 1,000 fish entering the river are previous spawners, having been derived from 440 spawning fish, giving a survival rate for both sexes of 9.4%, which agrees quite closely with the Wye figures mentioned previously.

Fishing for kelts was practised on the River Lee over a century ago, but even in those days it was recognised that the food value of these fish was slight. Fortunately, since that time enlightened opinion has changed with respect to the taking of kelts for food. As Mr. Hutton records, nature at no cost to ourselves transforms small quantities of almost inedible fish into quantities of "most excellent and nourishing diet" *Fishing Gazette* (No. 3,496, Vol. 126, p. 197). He gives an example of a kelt weighing 8 lb. transformed in 15 months to a clean 28 lb. fish. In Ireland over a period of years a large number of stripped fish (kelts) have been marked at the various hatcheries. The results given by Hillas, 1905, and those obtained since 1915 have been combined in Table 5.

TABLE 5

| | RIVER | | | | | | | | Total all Rivers |
|--|-------|-------|--------|------|----------|-------|----------------------|--------|------------------------|
| | Bann | Foyle | Owenea | Erne | Aasleagh | Laune | Blackwater (Cork) | Slaney | |
| Number of fish examined | 15 | 14 | 96 | 5 | 1 | 5 | 2 | 1 | 139 |
| Total Weight of Kelts in lb. | 75 | 66½ | 531 | 26 | 11½ | 26 | 16¼ | 11 | 763½ |
| Total Weight of clean fish in lb | 170 | 136 | 1,022 | 54½ | 18 | 61½ | 30 | 27 | 1,418½ |
| Increase in weight (per- centage) | 127 | 105 | 93 | 108 | 57 | 136 | 85 | 145 | 98 |

The total shows that the kelts after recovery weighed almost double their original weight, apart from any question of the quality of the resultant material. Surely this alone furnishes a good reason for the protection of the kelt

That the proportion of females which survive spawning is greater than that of the males is well known, but the fact that some male kelts do survive to return for a second time as clean fish is obvious from the marking of kelts which has been carried on in many places. In the Owenca in County Donegal 2,875 kelts have been marked in the past twelve years. Of these 1,969 were females and 906 males. The recaptures of clean marked fish totalled 127, of which 106 were females and 21 males. The survival rate of the females compared with the males from these results is 2.35 to 1.

Mr. Hutton, in the article in the *Fishing Gazette* already referred to in another connection, stated by way of conclusion "Therefore I have no hesitation in saying that kelts are well worth preserving, and that it would be a most stupid mistake to make it legal to kill them, for even the best 'mended' kelt is most unpalatable food and provides very little real nourishment." The results of the present preliminary investigation confirm Mr. Hutton's views, and show clearly *why it is illegal to kill salmon kelts*. It is hoped to repeat the investigations in future years so that we might have, in relation to Irish salmon, some idea of the proportions and fluctuations in the numbers of previously spawned fish. In this matter, however, the co-operation of the owners of the various fisheries is essential. Fortunately most of the owners of important commercial fisheries in Ireland have been ready to give us every assistance in these investigations, and it is not anticipated that there will be any change in this respect in future years

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No. 2.

OBSERVATIONS ON *GIBBERELLA SAUBINETII* (MONT.) SACC. ON
CEREALS IN IRELAND IN 1943 AND 1944

By

ROBERT MCKAY, D.Sc., AND JAMES B. LOUGHNANE, M.Sc.,
DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY COLLEGE, DUBLIN.

(Plate I.)

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FOLLOWING the severe outbreak of *Gibberella Saubinetii* on wheat in this country in 1942 (4), observations on the occurrence and prevalence of this fungus on cereals were continued in 1943 and 1944. Some investigations were also carried out at the Albert Agricultural College, Glasnevin, on the development of "Seedling Blight" and on the general infection of the host. The results are presented in the accompanying paper.

NATURAL OCCURRENCE OF "SEEDLING BLIGHT" OF WHEAT IN 1943.

Failures of wheat brairds were reported as being rather common in the spring of 1943, the worst misses occurring in crops sown in February, March, and April. A number of samples of the seed sown and small seedlings from infected fields in several counties were examined. In all cases investigated the specimens came from fields in which the brairds were exceptionally poor. Many of the ungerminated grains which were collected in these fields were reddish or carmine in colour, and were found to be permeated throughout with the fungus *Gibberella Saubinetii*. The specimens of plants received at the same time were small, chlorotic, and their root system poorly developed. Many of those roots present were brown and rotting, and some of the seedlings were dead and others dying. Incubation of these plants under moist warm conditions, in the majority of cases, gave conidial fructifications of *G. Saubinetii*. There is no doubt that the poor brairds in these cases of spring-sown wheat were mainly due to using badly infected seed. Furthermore the investigations established the facts that "Seedling Blight" of wheat occurred in

the following counties, Limerick, Clare, Westmeath, Offaly, and Dublin, and that this phase of disease caused by *G. Saubinetii* is one which must be reckoned with, especially in seasons following wet years when scabbed wheat has been prevalent. Moreover, plants which have been killed off by "Seedling Blight," later in the growing season are a potential source of infection for "Ear Blight," as will be shown further on

THE OCCURRENCE AND PREVALENCE OF "EAR BLIGHT" AND "SCAB" ON CEREALS IN 1943.

The term "Ear Blight" is that applied to infected cereal heads where only the *Fusarium* stage of the fungus is present, whereas when "Scab" is used it implies the presence of perithecia containing ascospores of *Gibberella Saubinetii*

As a result of instructions issued by the Department of Agriculture to the various Instructors and Overseers in Agriculture, 82 samples of wheat (in ear) and 1 sample of threshed grain were submitted for examination in 1943, as being suspected of "Ear Blight" or Cereal "Scab". Of these, 62 samples showed the presence of *G. Saubinetii*, 26 of them showing only the conidial stage of the fungus, and 36 having well-developed perithecia containing ascospores. This "Scab" stage of the disease was again forwarded from 10 different counties, but 6 of these constituted new records for perithecia, namely counties Mayo, Monaghan, Meath, Dublin, Kilkenny, and Waterford. Reports received along with the specimens, stated that the disease was not nearly so virulent along the western seaboard as in the previous year. The disease was reported from Co. Kilkenny as present in about 60 per cent. of the wheat crops, but the damage was not serious; and in Co. Cavan it was reported as occurring in almost every crop of the variety Queen Wilhelmina. These two counties are particularly mentioned as being those from which the disease was reported as being most prevalent.

Poor braids, followed naturally by thin stands due to bad germination of infected grain and to "Seedling Blight," undoubtedly reduced yields in some cases. However, apart from these, and notwithstanding the general occurrence of infection in many wheat crops at harvest time, there was no record of any serious reduction in yield due to the "Ear Blight" and "Scab" stages of the fungus, such as occurred in 1942. This was borne out by examination of infected heads. With one exception, the grain in even the worst diseased heads was well developed, and although traces of pink-coloured grains were obvious in many harvested crops, shrivelling of grains was almost negligible, and germination was not seriously impaired by the amount of infection present.

The only other cereal forwarded with *G. Saubinetii* on it in 1943 was barley, a single sample being received which was badly infected with the perithecial stage of the fungus. This is the first record of "Scab" on barley in Ireland.

THE OCCURRENCE OF *GIBBERELLA SAUBINETII* ON CEREALS IN 1944

Only one case of "Seedling Blight" caused by *G. Saubinetii* was identified in the spring of this year, the affected specimens being received from Co. Monaghan.

Through the continued co-operation of the Department of Agriculture, suspected specimens of cereals were again forwarded for examination. These included 92

samples of wheat, 20 samples of oats and 2 of rye. Considering the wheat first, 63 samples of this cereal were found infected with *G. Saubinetii*, 45 of these having the *Fusarium* stage only but the other 18 showed "Scab" in addition. On these scabbed specimens the perithecia, while well developed and mature, were not nearly so numerous as on infected heads in the two previous years. At the same time, wheat ears with perithecia were received from 9 counties; Co. Donegal being added to the existing records. This brings up to 17 the total number of counties from which perithecia have been recorded during the past three years. In the case of the oats, 8 of the 20 samples were infected with *G. Saubinetii*, 5 of them showing the "Ear Blight" stage only, and the other 3 having perithecia in addition. The two samples of rye showed only the *Fusarium* stage of the fungus.

Although samples of cereals were received from 17 counties in 1944, infection with *G. Saubinetii* was only detected on specimens from 12 of these counties. It was found that in cases where "Ear Blight" only was present on wheat heads (*i.e.* the conidial stage of the fungus), perithecia could be induced to develop in many cases by simply cutting the attached culms to a length of 6 inches and standing these with their cut ends in water in an open glass beaker, the heads projecting slightly above the mouth of the vessel. Where this was done at ordinary room temperature perithecia often developed, not however on the ears but on the stems just above the surface of the water.

VARIETIES OF WHEAT FOUND INFECTED BY *G. SAUBINETII* IN 1943 AND 1944.

| | | | |
|------------------|----------|---------------------|---------|
| Queen Wilhelmina | 58 crops | Yeoman .. | 2 crops |
| Atle | 14 " | Pajbjerg | 2 " |
| Red Marvel | 9 " | Squarehead's Master | 1 " |
| Diamant | 6 " | Flygia | 1 " |
| April Red | 4 " | Not named | 24 " |
| Manitoba | 4 " | | |

CROP PRECEDING DISEASED WHEAT.

Previous cropping and seed treatments before sowing are shown by the following data in so far as these details were supplied in the two years for affected crops:

| | | | |
|-------------------|------------|------------|------------|
| Potatoes | 30 fields. | Oats | 14 fields. |
| Mangels and Sugar | | Barley | 1 " |
| beet | 7 " | Lca | 6 " |
| Other roots | 15 " | No details | 45 " |
| Wheat | 7 " | | |

SEED TREATMENT BEFORE SOWING.

| | | | |
|-----------------|----------|----------------------|----------|
| Agrosan | 4 crops. | Mercurial dusts, un- | |
| Ceresan | 8 " | specified | 10 crops |
| Carbosan | 3 " | Copper sulphate | 9 " |
| Coversine | 2 " | Copper carbonate | 1 " |
| Harvesan | 1 " | Tar | 1 " |
| Golden Grain | 1 " | No treatment | 38 " |
| Farmer's Friend | 1 " | No information | 46 " |

CLIMATIC CONDITIONS IN 1943 AND 1944

The spring in both these years was abnormally dry. Thus in 1943 rainfall for the month of February was only 0.99 inch and for March 0.72; in 1944 the relative figures were 1.19 and 0.30. Precipitation for April each year was also very low, see accompanying data on rainfall. These figures refer to Co. Dublin, but similar drought conditions were general throughout the country. Both of these dry springs were followed by dull cloudy summers, with just a little more sunshine for the months of July, August, and September, than in 1942, this increase being 10 hours in 1943 and 6 hours in 1944. Fortunately, in both years a spell of relatively dry weather coincided with the flowering of the wheat and the subsequent soft dough stage. The result of this was that climatic conditions were not favourable for the fungus at the most critical period of the host's growth. Hence, although the disease was prevalent enough in many crops towards harvest, there was no serious reduction in yield nor excessive shrivelling of grain in either year, such as occurred in the epidemic of 1942 (4).

The number of days on which precipitation occurred, and rainfall for each month, April to September inclusive, are shown at four separate stations in 1943 and 1944.

| MONTH | | | Glasnevin, Co. Dublin (East Coast) | | Ballyhaise, Co. Cavan (North Mid-lands) | | Athenry, Co. Galway (West Coast) | | Clonakilty, Co. Cork (South Coast) | |
|------------|------|-----|--|--------------------|---|--------------------|--|--------------------|--|--------------------|
| | | | No of days | Rainfall inches | No. of days | Rainfall inches | No of days | Rainfall inches | No. of days | Rainfall inches |
| April, | 1943 | .. | 16 | 1.07 | 11 | 1.55 | 9 | 1.22 | 9 | 2.01 |
| May, | " | . | 16 | 4.10 | 19 | 3.16 | 15 | 3.47 | 16 | 4.77 |
| June, | " | ... | 17 | 2.10 | 21 | 3.65 | 18 | 3.93 | 18 | 3.03 |
| July, | " | ... | 15 | 1.58 | 14 | 2.88 | 15 | 2.81 | 13 | 2.73 |
| August, | " | .. | 21 | 2.53 | 25 | 6.30 | 25 | 6.43 | 24 | 3.51 |
| September, | " | . | 24 | 2.57 | 21 | 2.72 | 26 | 2.95 | 20 | 3.33 |
| TOTALS | | ... | 109 | 13.95 | 111 | 20.26 | 108 | 20.81 | 100 | 19.38 |
| April, | 1944 | . | 14 | 1.69 | 8 | 2.11 | 10 | 1.82 | 13 | 3.26 |
| May, | " | .. | 10 | 0.92 | 14 | 2.15 | 11 | 3.23 | 9 | 0.98 |
| June, | " | .. | 16 | 1.46 | 18 | 2.38 | 17 | 3.01 | 12 | 1.12 |
| July, | " | ... | 20 | 2.37 | 22 | 3.86 | 23 | 4.50 | 20 | 4.22 |
| August, | " | . | 16 | 2.61 | 15 | 3.51 | 21 | 3.41 | 13 | 1.92 |
| September, | " | ... | 18 | 5.56 | 19 | 4.31 | 17 | 4.15 | 18 | 4.98 |
| TOTALS | | .. | 94 | 14.61 | 96 | 18.32 | 99 | 20.12 | 85 | 16.48 |

INVESTIGATIONS ON "SEEDLING BLIGHT" AT THE ALBERT AGRICULTURAL COLLEGE.

Experiment 1.—In the spring of 1943 wheat grain obviously infected with *G. Saubinetii* was mixed with healthy grain, and sown in pots of sterilized soil in an unheated greenhouse. No plants developed from any of the infected grains. Evidently this diseased seed was too thoroughly permeated by the fungus to germinate. On the other hand, all the healthy grains gave rise to normal plants, and there was no evidence of any spread of the fungus in the soil from the diseased grains to the healthy plants.

Experiment 2.—On the 6th April a nine-day-old culture of *G. Saubinetii* was mixed with the top two inches of sterilized soil in pots. These pots were sown with 240 grains of Queen Wilhelmina wheat which had been surface sterilised for five minutes in mercuric chloride solution 1/1,000, and then rinsed in sterile water. The pots were kept in an unheated greenhouse. The emergence of the plants from the soil was irregular, twelve days after sowing 208 plants had appeared, and at the end of three weeks the final number was 232. Of these, 60 were weak and chlorotic, and 2 dying. The latter succumbed inside the next week through basal decay, and the majority of the chlorotic plants showed a similar decay of the lowest leaf sheath, with a tendency for the rot to pass into the crown of the plant. When some of these plants were pulled up, the root system was found to be poorly developed and rotting, Fig. 1, Plate 1. Six weeks after sowing the number of chlorotic plants was 64, and 4 more had died after reaching a height of $2\frac{1}{2}$ inches. These four were affected by severe root rot. The two seedlings which had first succumbed were now found *in situ* to have well developed perithecia of *G. Saubinetii* on their dead tissues above ground Figs. 1 and 2, Plate 1, and these were exuding ascospores by the middle of June. Incubation in a humid atmosphere of the remaining dead specimens induced perithecial development here also in about a month. Measurements of the perithecia found on these seedlings showed them to be somewhat smaller as a rule than those formed on the glumes (4), the maximum being $219 \times 175\mu$, minimum $175 \times 131\mu$, with an average of $197 \times 146\mu$.

No further dying of young plants occurred. The weak chlorotic individuals recovered and grew away more or less from the disease, but later in the season the fungus *G. Saubinetii* was obtained readily enough from dark brown areas in their outer leaf sheaths near the soil level. These plants were subsequently badly attacked by mildew, *Erysiphe graminis*, and quite a number of tillers failed to produce heads and died prematurely. Examination of these dead tillers in the month of October revealed their basal parts to be completely rotted, and numerous perithecia of *G. Saubinetii* present just above ground. In addition, on other tillers there were a few heads which failed to produce any grain, the ears being bleached and resembling those of plants attacked by *Ophiobolus graminis*. This failure may have been caused by the severe attack of mildew concurrently with the rather restricted root run of the plants, but it may be significant that occasional perithecia of *G. Saubinetii* were found on the base of these unproductive culms.

Appropriate controls sown in uninoculated sterilized soil and kept under similar conditions remained free from *G. Saubinetii* throughout.

Experiment 3.—Another attempt was made to induce "Seedling Blight" under glasshouse conditions by sowing 60 grains of Pajbjerg and 60 of Desprez in pots of sterilized soil. In this case the grain was sown on the 10th June and the soil in the pots then sprayed with a suspension of ascospores of *G. Saubinetii*. Germination of the grain was quick and growth of the plants at this time rapid. No "Seedling Blight" developed. These plants also became attacked by *Erysiphe graminis*, and after they reached the height of 24 inches the pots were placed on the floor of the greenhouse, where they remained until the end of the season. Prior to being thrown out in October an examination of the base of the plants showed that quite a number of them had developed perithecia of *G. Saubinetii*, although no signs of disease due to this fungus had been noticed previously.

Experiment 4.—This was an endeavour to produce "Seedling Blight" outside. Two hundred grains of each of the varieties Atle, Pajbjerg, and Queen Wilhelmina, were steeped for half an hour in a suspension of ascospores of *G. Saubinetii*. These contaminated grains were sown outside in the month of July. After sowing, but before the grain was covered with soil, it was also sprayed with the spore suspension. The plants appeared rapidly and grew away vigorously. Again there was no "Seedling Blight" nor any visible appearance of infection with the fungus. At heading out however, on comparing the inoculated row of Atle with its control row alongside, it was seen that, although the number of tillers in each was approximately the same, the number of heads in the former was considerably less than in the control. By the end of September some of the tillers in the inoculated row had died, their bases being rotted. The death of these tillers could not with certainty be attributed to *Gibberella Saubinetii*, but when the basal portion were incubated in a moist chamber perithecia of this fungus developed and finally produced ascospores.

DOES *GIBBERELLA SAUBINETII* PERSIST IN THE SOIL?

A number of pots of soil from Experiment 2 above, in which wheat plants had developed "Seedling Blight" or basal rot in 1943, were kept. The diseased plants were cut off at soil level and the roots remained in the soil. In December 1943, the topmost inch of soil in these pots was loosened, disturbing the roots of the previous plants as little as possible. Healthy wheat grain of the variety Queen Wilhelmina was then sown in the pots. These were then divided into two batches, one of which was kept in an unheated greenhouse and the other in a heated glasshouse. The seedlings in the latter emerged ten days before those in the cool house. There was 99 per cent. germination, and all seedlings appeared without any trace of disease in 1944. The plants grew to maturity and produced heads well filled with grain, and at no time during the growth of these plants was there any symptom of disease. After harvesting the grain, the pots with their stubble were placed under the benches of the greenhouse, where they remained for two months. The bases of the plants were then examined but no trace of *G. Saubinetii* could be found.

The evidence obtained from this experiment indicates that soil which produced plants diseased with *G. Saubinetii* does not retain its infectivity until the following season, even though the remains of such infected plants have been added to it.

The reason for this may be the one suggested by Bennett (1), namely that under field conditions much *Gibberella*-infected material is rendered innocuous through competition with saprophytes.

THE RELATION OF ANTHERS TO INFECTION OF WHEAT EARS BY *GIBBERELLA*
SAUBINETII ON VAR *QUEEN WILHELMINA*

In June 1943, heads of wheat growing in a greenhouse were sprayed with a suspension of ascospores of the fungus when the anthers were extruded, and were then covered with small flasks to maintain a moist atmosphere. Four days later a white woolly growth of the fungus was visible on a number of anthers, but no other part of the ear showed infection at this time. Ten days after inoculation, 12 out of the 14 sprayed heads showed symptoms of disease. In all cases the anthers were the first to show signs of infection, and the disease then appeared on the glumes, which turned pale and developed brown patches. Infection soon spread to the rachis, and then the whole head rapidly bleached. No grain developed in any of the infected spikelets, but some shrivelled kernels were found in neighbouring spikelets.

Similar inoculations were carried out on 17 heads which had not yet come into flower. A few of these heads showed extruded anthers on the day following inoculation, and three days later 3 heads had anthers with a good growth of mycelium, but infection of the glumes was not apparent. Ten days after inoculation 5 heads were visibly infected, but only in three cases was growth of mycelium as profuse as when the anthers were exposed at time of spraying. The number of infected heads did not increase, and those already diseased developed symptoms similar to what are described above.

In another experiment the anthers were removed before extrusion from 12 heads of wheat growing in the open. These heads were then sprayed with a suspension of ascospores, and immediately covered with small flasks. Other heads which were in flower on the same plants were similarly inoculated and covered but no anthers were removed. Dealing with the latter lot first, as in previous cases, the anthers showed infection before other parts of the flower, and the disease then developed on the glumes. Three weeks after inoculation mycelium was abundant on these ears, being faint pink in colour and bearing sparse fructifications of the *Fusarium* type along the basal edges of the glumes. These infected heads remained slender, ripened prematurely, and all of them developed definite "Ear Blight". The only grain found in them consisted of a few shrunken kernels. In the case of the first lot, that of the emasculated heads, no definite evidence of infection could be obtained, and plump well-developed grains formed in the majority of the spikelets. From this experiment it would appear that the removal of anthers rendered the ears less liable to infection.

Emasculated and non-emasculated wheat heads growing in the open were also sprayed with a suspension of ascospores of *G. Saubinetii* but the heads were left uncovered. In neither case was there any development of "Ear Blight" up to the time of ripening. Similarly, when wheat heads both immature and almost ripe

growing in the greenhouse were sprayed with a suspension of ascospores and left uncovered no disease developed. Sufficient moisture was evidently not present in these cases to ensure infection

SPREAD OF "EAR BLIGHT" FROM DISEASED TO HEALTHY WHEAT EARS BY CONTACT.

In the month of August ten bundles of healthy wheat heads with stems 15 inches long were collected, all of these bundles were nearly ripe with the exception of the variety Atle, which was green at the time of collecting. Six of these bundles had heads of wheat badly infected with *G. Saubinetii* placed in their centre, and each bundle was tied so that it resembled a small sheaf. The bundles were then placed side by side on a bench in the laboratory. Four of those containing diseased heads and two without such heads were sprayed frequently with water for a period of three weeks, two bundles without diseased heads and two containing diseased ears being left unsprayed. When the bundles were opened and examined at the end of 45 days the following were the findings:

Bundle 1. Var. Atle.—Heads green and in flower when diseased ears were placed in the bundle; kept sprayed with water. A number of originally healthy heads in contact with diseased ones became infected and bound together by a web of mycelium. No grain was formed in either affected or unaffected heads.

Bundle 2.—Heads ripe at time of inoculation; kept sprayed. A number of heads on contact with diseased ones were joined to them by a mycelial web, but this was less profuse than in Bundle 1. Some of the grains in the newly-attacked heads were infected by the fungus.

Bundle 3.—Heads slightly green, grain well formed at time of inoculation, kept sprayed. Heads which were in contact with diseased heads attacked, and grains in affected portions of these heads shrivelled.

Bundle 4.—Heads nearly ripe, grain well formed at time of inoculation, kept sprayed. No infection developed on healthy heads even when in contact with diseased ones. Variety Desprez.

Bundles 5 and 6.—Uninoculated heads kept sprayed developed no symptoms.

Bundles 7 and 8.—Heads inoculated and kept dry developed no infection from diseased ears.

Bundles 9 and 10.—Heads uninoculated and kept dry developed no infection.

DISCUSSION.

Some investigators believe that a relatively high soil temperature is necessary for the development of "Seedling Blight" of wheat (2 & 3). Bennett (1) however has shown that it can develop at a low soil temperature, particularly when environmental conditions are unfavourable for the host. The occurrence of "Seedling Blight" naturally in the spring of 1943 confirms Bennett's work. This phase of disease caused by *Gibberella Saubinetii* was reported mainly from sowings of wheat

made from February onwards. Two factors were probably involved in the prevalence of the disease in wheat sown at this time when compared with earlier sown crops (i.e., autumn sown). These are, first, the best wheat is usually harvested and threshed early, and this grain is available and largely used for autumn sowing, whereas inferior crops are not threshed until later, and more infection is liable to be present. The second factor was climatic conditions, wheat brairds suffered badly from harsh dry weather in 1943, and this favoured the disease.

Grain which was obviously infected by *G. Saubinetii* failed to grow even under the most favourable conditions in a greenhouse. It is probable, therefore, that plants affected by "Seedling Blight" in the field only arise from grain which is slightly infected.

Under experimental conditions in the greenhouse with grain sown in contaminated soil in April, the percentage of plants killed by "Seedling Blight" was low. Conditions for growth at this time were good, and it is reasonable to assume that many more affected plants would succumb under more adverse conditions. This is actually borne out by field observations. Plants which are killed by the fungus in the seedling stage are a potential source of infection for the standing crop later on, as perithecia are produced on their dead tissues, and these fructifications may be liberating ascospores by the time wheat comes into flower. Under favourable climatic conditions many plants which are attacked by "Seedling Blight" may grow out of the disease more or less. Such plants are also a potential source of "Ear Blight," because infection is still present on the base of the culms, and in addition to causing dead or sterile tillers, perithecia may be formed.

A high atmospheric humidity is requisite for the development of "Ear Blight," and if such coincides with the flowering period of the host, then the greatest damage occurs. The fungus grows freely on the anthers as Pugh *et al.* (5) have pointed out. As the wheat ripens the ears become more resistant to the disease, but infection may occur in stooks if sufficient moisture is present. Abundant moisture is also essential for the formation of perithecia, and the prevalence of these fructifications during 1943 and 1944 is attributed to the wet seasons. The importance of moisture for the development of perithecia is illustrated by their production on culms of wheat ears affected by "Ear Blight," when they are kept standing in water, and also by their formation on dead seedlings and tillers of affected plants.

SUMMARY.

As a sequence to the epidemic of *Gibberella Saubinetii* in 1942, a considerable amount of infected grain was used for seed purposes the following season, none other being available. This resulted in a number of cases of thin brairds due to bad germination of the seed and to "Seedling Blight".

"Seedling Blight" was produced experimentally under glasshouse conditions, by sowing wheat in sterilized soil inoculated with a pure culture of *G. Saubinetii*. Plants killed by "Seedling Blight" are a potential source of infection to the standing crop as they may produce perithecia. Similar fructifications may be formed on the base of plants which recover from "Seedling Blight" attacks.

"Ear Blight" and "Scab" were both rather prevalent on wheat in 1943 and 1944. The approximate percentage of affected crops showing perithecia each year was 58 and 28 respectively. No serious reduction in yield nor shrivelling of grain took place in either year as climatic conditions favoured the host at its most critical period, and the fungus attacks occurred late in the season.

EXPLANATION OF PLATE I.

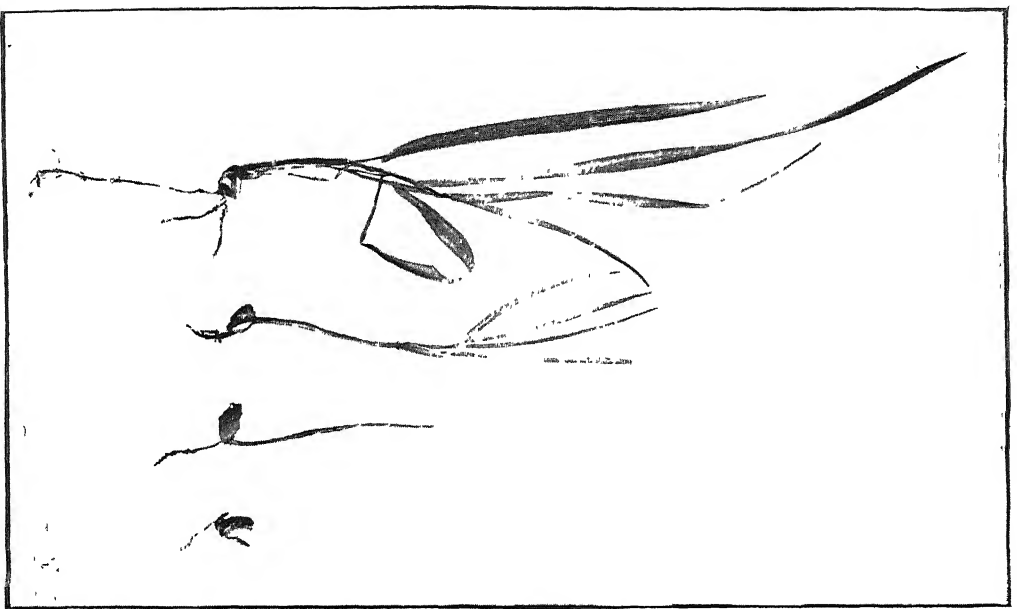
Fig. 1.—Wheat seedlings variety Queen Wilhelmina showing various stages of "Seedling Blight," obtained by adding a pure culture of *Gibberella Saubinetii* to sterilized soil before sowing. Note: perithecia are already developing about half way up on second seedling from right.

Fig. 2.—Enlarged view of perithecia of *G. Saubinetii* on dead tissues of young plant killed by "Seedling Blight" ($\times 6$).

Fig. 3.—Healthy control plant showing well-developed root system, the same age and kept under the same conditions as plants in Fig. 1, but grown in uninoculated soil.

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Phadus b1

Fig. 1.



Fig. 2.

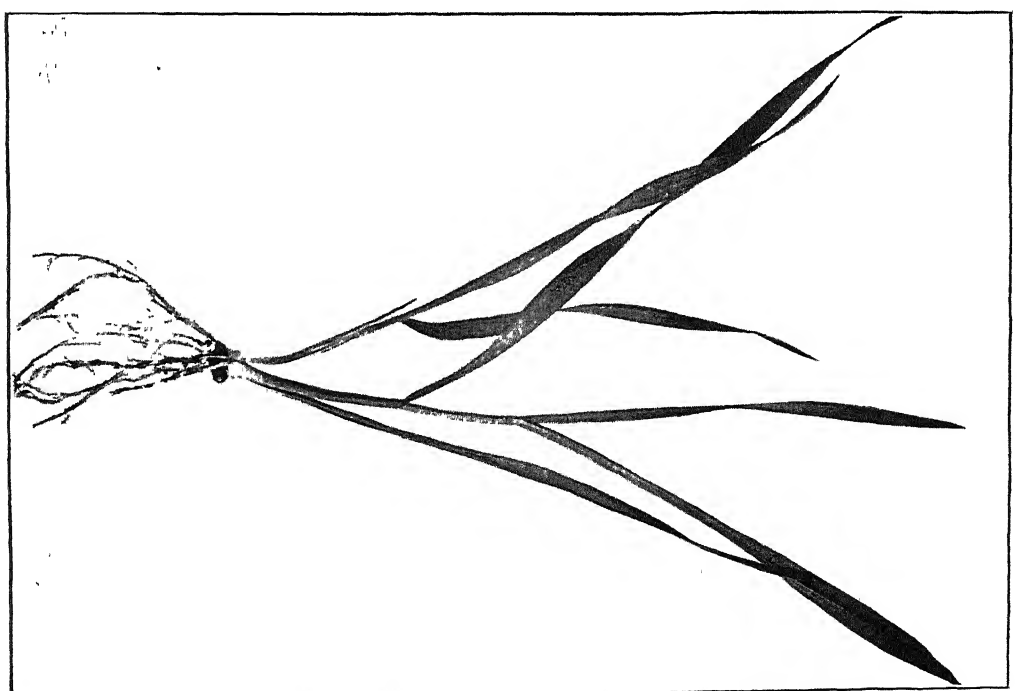


Fig. 3.

[G. H. McLEAN.]

No. 3.

CONTRIBUTIONS TOWARDS AN UNDERSTANDING OF THE CALCICOLE
AND CALCIFUGE HABIT IN SOME IRISH PLANTS.

By

D. A. WEBB, PH.D. AND ANNE V. HART, M.Sc.

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THE problem of the explanation of the calcicole and calcifuge habit in plants is still, in spite of the large amount of attention it has received, largely unsolved. A number of theories have been put forward, each with some evidence in its favour, but it is uncertain to what extent they should be regarded as mutually exclusive and to what extent as complementary; and it is also possible that the various factors suggested may in some cases operate simultaneously on the same plant, whereas in other cases different species in the same community may find themselves affected by different features of the same environment. The experiments and observations described below do not profess to form anything more than a small contribution towards the solution of this complex problem, and we would wish our results to be regarded as suggestive rather than conclusive. But their publication at this stage is, we feel, warranted by two considerations: firstly, that whereas most recent work has centred on the analysis of the calcifuge habit we have devoted more attention to calcicole species, and it is in relation to them that the more interesting results have been obtained; and secondly, that hitherto no study of the problem has been made with specific reference to Irish species and to the physico-chemical conditions that obtain in Irish soils.

GENERAL CONSIDERATIONS.

It must be explained at the outset that the words "calcicole" and "calcifuge" are here used in a purely descriptive sense. A calcicole plant is one which, under normal and natural conditions, is rarely or never found growing except in a soil that contains calcium carbonate in considerable quantities; a calcifuge plant one which, under normal and natural conditions, is rarely or never found growing except in a soil that is virtually free from calcium carbonate. The terms are in no way intended to beg the question whether or not the calcium content of the soil is in itself the critical factor; and though for some species the terms "silicicole" or "oxyphil" may be justified as alternatives to "calcifuge" they are, in the present state of our knowledge, not so well suited to a general discussion of the problem.

The chief points in which calcareous soils differ from non-calcareous ones, and which may, therefore, be of importance in determining the calcifuge or calcicole habit, are as follows:—

(i) *Alkalinity*.—The pH of calcareous soils in this country is usually between 7.0 and 8.5; that of non-calcareous soils usually between 4.0 and 6.5. There

is no evidence in the literature to suggest that a moderate degree of acidity is in itself injurious to calcicole species. On the other hand it has been claimed by Mevius (1924) for *Sphagnum spp.*, and by Rayner (1921) for *Calluna vulgaris*, that these calcifuge species require an acid medium for healthy growth. Pearsall (1922, 1926) however, who has found the latter species growing naturally on neutral gravels, disputes this conclusion.

(ii) *Higher calcium content.*—Many acid soils contain very little available calcium; and De Silva (1934) has shown that certain species which possess a rather irregularly calcicole habit will flourish on any soil, no matter what its acidity, if the available calcium content is high enough. This interpretation of the calcicole habit has also been suggested by Truog (1918), Lundegardh (1924), and others. It has also been suggested that calcifuge species are adversely affected by a high concentration of calcium, which interferes with the absorption of potassium or magnesium (see Salisbury, 1920).

(iii) *Lower basic ratio.*—According to Pearsall (1922, 1926) and Pearsall and Wray (1927) neither acidity nor a low calcium concentration in absolute units is as important for calcifuge species as is a high ratio of univalent cations ($\text{Na}^+ + \text{K}^+$) to bivalent ($\text{Ca}^{++} + \text{Mg}^{++}$). This ratio is, of course, normally high in acid, and low in calcareous soils.

(iv) *Precipitation of the sesquioxides.*—The amount of iron and aluminium in solution in the water of an acid soil is often considerable; in a calcareous soil it is very small. This arises directly from the difference in pH, and also, in the case of iron, from the oxidation in calcareous soils (which have usually a much higher redox potential than acid ones) of ferrous to the much less soluble ferric hydroxide. It has been held, therefore, that calcicole species are those that are especially susceptible to aluminium poisoning, and that calcifuge species are those that have an unusually high iron requirement.

(v) *Lack of organic toxins.*—Whether peaty soils and the waters derived from them contain soluble organic compounds that are, quite apart from the acidity with which they are usually associated, toxic to certain species, is still a very vexed question. But it is possible that this may be a factor which prevents calcicole species from growing on certain acid soils.

(vi) *Higher nitrogen content.*—Calcareous soils are usually richer in nitrates than are non-calcareous: see Salisbury (1920), and Lundegardh (1931) for references. It has been suggested therefore that some calcicole species are really nitrophiles.

(vii) *Physical factors.*—In the British Isles most calcareous soils are better drained, and therefore drier, warmer, and better aerated than non-calcareous ones. In Ireland this contrast is of great importance, since the calcicole flora is best developed on the limestone pavements of the west and the eskers of the central plain—both of them environments which are, compared with the rest of the country, exceptionally well drained. Certain apparent calcicoles may, therefore, really be dry-soil plants.

In any attempt at an experimental analysis of the calcicole and calcifuge habits it is of course desirable to dissociate as far as possible the influence of these several factors. But this problem presents, unfortunately, grave difficulties. It is necessary to realize that whereas some of these factors are accidentally, and therefore separably, associated with alkalinity of the soil, others are inevitable concomitants of it. To the former category belong (vi) and (vii), and it is relatively easy to eliminate their influence in experimental cultures. The same is true of (v) if it is supposed that the toxins act independently of the acidity, but authors are not agreed on this point, and as there seems to be little reliable information on the matter we shall not further consider this factor.

Factors (i) to (iv), on the other hand, cannot be completely dissociated from differences in acidity. It is possible to prepare acid soils in which the calcium content and the basic ratio vary within wide limits; and also, if water cultures be used, to vary the concentration of dissolved iron and aluminium in an acid medium. But to bring about the corresponding variation in an alkaline soil or solution is impossible. If any substance other than calcium carbonate is used to maintain the alkalinity new and disturbing factors are introduced; and calcium carbonate inevitably means plenty of calcium in solution and a high basic ratio. Moreover the sesquioxides are inevitably precipitated by a rise in pH, no matter what the composition of the medium.

While we claim, in the experiments described below, to have largely eliminated factors (vi) and (vii), no attention has been paid to (iv) or (v). In some of the experiments a partial dissociation of (ii) and (iii) from (i) has been attempted.

EXPERIMENTAL CONDITIONS.

Plants belonging to 22 species that are, in Ireland, either calcicole or calcifuge were grown from seed in pots or boxes in a cool greenhouse. Two series of cultures were made of each species; these differed from one another in one particular only—the amount of calcium carbonate in the soil. Care was taken to see that in all other physical and chemical conditions, as well, of course, as in the origin and quantity of the seed sown, the two series of cultures were identical. In some cases only a single sowing on each soil-type was made; in other cases the sowings were duplicated or triplicated. But in either event the results presented here represent the average result given by a considerable number of seedlings, whether grown in the same box or not—a number large enough to eliminate the effects of fortuitous genetic variation in the material. The species discussed below all gave reasonably consistent results; the rather inconsistent results given by three other species have been omitted.

The seed was collected from wild plants growing in their most typical environment. In all cases seed from several separate individuals was mixed together, and later distributed at random among the different cultures.

The basis of the artificial soils used for the experiments was a peat-sand mixture which consisted of 22% (by weight) of air-dry peat and 78% of sea sand, freed from calcium carbonate by treatment with acid. For some of the experiments the sand was supplemented by debris derived from well-weathered granite. Both the peat and the granite debris presumably contained small quantities of soluble mineral matter, but since the aim of the experiments was to control only the supply of calcium carbonate, and to provide an adequate supply of all other nutrients, this has no bearing on the comparative results presented here.

The peat-sand mixture served without further addition as the soil for the non-calcareous cultures. For the calcareous cultures calcium carbonate was added in the form of a mixture of equal parts of precipitated calcium carbonate powder and coarsely ground oyster shells. In most of the experiments enough of this mixture was added to form 13.3% of the weight of the air-dry soil; but for those species numbered (4), (5), (6), (9), (10), (13), (14) and (21) in the list below, only half this quantity (i.e. 6.7% of the total weight) was used. In either case the soil was distinctly alkaline; the change was made because it was felt that the conditions provided by the larger proportion of calcium carbonate were perhaps rather too extreme. They can, nevertheless, be matched in many natural soils.

A stock nutrient solution was made up; it contained 0.13 M calcium nitrate, 0.29 M potassium dihydrogen phosphate, 0.35 M magnesium sulphate, and traces of iron, copper, zinc, manganese and boron. This was diluted 40 times before use. It was given every one or two weeks, in quantities determined by the rate of growth of the plants; but the same quantity was, of course, given on each occasion to all the cultures of any one species. All plants were given water as required, the only criterion being the appearance of the soil. They were thinned as soon as this became necessary to prevent serious over-crowding.

The pH of the non-calcareous soil (i.e., the peat-sand mixture with nutrient solution) was about 5.2, that of the calcareous soil about 8.0.

In some of the experiments the cultures in non-calcareous soil were duplicated: one series was given the nutrient solution just described, the other a similar solution but with the calcium nitrate replaced by an equivalent of sodium nitrate. The purpose of these experiments was to assess the importance for growth in an acid soil of a plentiful calcium supply.

The following species of plants were grown:—

CALCICOLES.

- | | |
|---|---|
| (1) <i>Geranium lucidum</i> L. | (9) <i>Poterium sanguisorba</i> L. |
| (2) <i>Carlina vulgaris</i> L. | (10) <i>Aquilegia vulgaris</i> L. |
| (3) <i>Blackstonia perfoliata</i> Huds. | (11) <i>Reseda luteola</i> L. |
| (4) <i>Origanum vulgare</i> L. | (12) <i>Centaurea scabiosa</i> L. |
| (5) <i>Juncus inflexus</i> L. | (13) <i>Erigeron acer</i> L. |
| (6) <i>Verbascum thapsus</i> L. | |
| (7) <i>Hypericum perforatum</i> L. | (14) <i>Antennaria dioica</i> Gaertn. |
| (8) <i>Leontodon hispidus</i> L. | (15) <i>Leontodon taraxacoides</i> Lac. |

CALCIFUGES.

- | | |
|------------------------------------|---|
| (16) <i>Juncus squarrosus</i> L. | (20) <i>Digitalis purpurea</i> L. |
| (17) <i>Radiola linoides</i> Roth. | |
| (18) <i>Senecio sylvaticus</i> L. | (21) <i>Rumex acetosella</i> L. |
| (19) <i>Jasione montana</i> L. | (22) <i>Lepidium heterophyllum</i> Benth. |

In these lists the species have been arranged in groups, in accordance with the extent to which the calcicole or calcifuge habit is strongly or feebly developed. Thus (1) to (3) are very strongly calcicole, and (14) and (15) only feebly so; similarly with the calcifuge species. Within each group the order is arbitrary. The necessary data for this grouping has been derived from *Cybele Hibernica* (2nd edition), Scully (1916) and Praeger (1933), supplemented by field observations. This gradation in the intensity of the calcicole or calcifuge habit should be borne in mind in interpreting the results given below.

RESULTS.

(a) *Total failure*—In only three cases did a plant completely fail to establish itself on one of the soils. *Blackstonia* failed on the non-calcareous, and *Juncus squarrosus* and *Radiola* on the calcareous soil. In all cases the seeds germinated normally, but the seedlings made no progress, and died within a fortnight of germination.

All these instances of failure are, naturally enough, of plants grown on the type of soil on which they are never found wild. But the case of *Blackstonia* is peculiar, since, as will be seen below, all the other calcicole species did well on non-calcareous soil. It is possible that the exceptional behaviour of this species may be connected with its mycorrhizal habit.

(b) *Difference in the rate of germination*.—In species (1) and (2) germination was much slower (by 6–12 days) on the calcareous than on the non-calcareous soil. This is striking in view of the particularly pronounced calcicole habit of these species. The same delaying effect of calcareous soil was shown to a lesser extent in species (6), (16) and (17).

Borthwick (1936) found that calcium, even in quite small concentration, delays germination in *Hypericum perforatum*, apparently by diminishing the permeability of the testa. In our experiments with this species germination was simultaneous on all soils, but it is probable that in none of them was the concentration low enough to bring about the rapid germination obtained by Borthwick in distilled water.

(c) *Difference in the percentage of seeds germinating*.—In species (1), (11) and (12) among the calcicoles, and (16), (18), (19) and (20) among the calcifuges, the number of seeds that ultimately germinated was significantly lower on the calcareous than on the non-calcareous soil. That this phenomenon should be shown by four out of five of the pronouncedly calcifuge species is not surprising: that it should be shown also by three calcicoles is less expected. The percentages of the seeds of these species (*Geranium lucidum*, *Reseda luteola* and *Centaurea scabiosa*) that ger-

minated on calcareous and non-calcareous soil respectively are as follows: 37% and 75%; 43% and 69%, 33% and 54%

Only one species (*Hypericum perforatum*) showed a significantly higher percentage of germination on calcareous soil. In the remaining species the percentage germinating on the two soils was almost identical.

(d) *Pathological symptoms*—In four species culture on calcareous soil produced symptoms that may fairly be described as pathological, in contrast to the normal appearance presented by the plants grown on non-calcareous soil. Two of these species were calcicole and two calcifuge. In *Reseda luteola* a chlorosis of the leaf-margins appeared about three weeks after germination, but after a further two weeks it disappeared. In *Centaurea scabiosa* the growth and geotropic response of the radicle seemed to be deficient on calcareous soil, with the result that most of the seedlings were not adequately rooted in the soil during their first few weeks. In the calcifuge species, *Senecio sylvaticus* and *Jasione montana*, the symptoms were not so definite, but for about a month the seedlings on calcareous soil looked extremely miserable, and at any time throughout this period their death would not have been surprising. A considerable proportion of the *Jasione* seedlings did, in fact, die, but the remainder ultimately made a fair recovery, as did almost all those of *Senecio sylvaticus*.

(e) *Different growth-rate in early stages*.—The most general result of the experiments is the smaller growth-rate of almost all the species on calcareous soil. *Blackstonia*, as has already been mentioned, failed completely on the non-calcareous soil. Two other calcicole species—*Origanum vulgare* and *Poterium sanguisorba*—showed throughout the course of the experiment identical behaviour on the two soils. But the remaining 19 species, calcicole and calcifuge alike, all showed slower growth in their early stages on the calcareous soil. In accordance with their later behaviour these species can be divided into four groups:—

(i) Those in which the plants on calcareous soil never recovered from this initial handicap and were still, after 6 or 12 months, very much smaller than the plants on non-calcareous soil. To this group belong species (2) and (14) (calcicole), and (19) and (21) (calcifuge), as well, of course, as the two calcifuge species mentioned earlier which completely failed on the calcareous soil.

(ii) Those that showed a partial, but not complete recovery from the initial handicap. To this group belong species (8) and (18).

(iii) Those in which the plants on calcareous soil recovered completely in the later part of the experiment, so that at the end they showed no significant differences from the plants grown on non-calcareous soil. To this group belong (5), (10) and (12) (calcicole), and (20) and (22) (calcifuge).

(iv) Those in which the recovery was more than complete—that is to say in which the plants on calcareous soil were at first smaller, but ultimately larger, or stronger and healthier than the others. It must be emphasized, however, that this reversal, though unmistakable, was in no case dramatic; for in all species the plants

on non-calcareous soil remained reasonably healthy and well-developed throughout. To this group belong species (1), (6), (7), (11), (13) and (15), all calcicole.

A few quantitative data may be given here, to illustrate in greater detail the behaviour of species in each of these four groups; to avoid repetition only one species will be selected from each group. C indicates calcareous, and NC non-calcareous soil; the dates indicate the time after sowing. The measurements indicate, of course, average dimensions, not those of any one plant.

(i) *Carlina vulgaris*.—6 weeks: C, plants 14 mm. across, with 2 leaves; NC, plants 27 mm. across, with 3-4 leaves. 10 weeks: C, plants 20 mm. across, with 3-4 leaves; NC, plants 50 mm. across, with 6-7 leaves. 26 weeks: C, 33 mm. across; NC, 100 mm. across. 54 weeks: C, 55 mm. across, NC, 140 mm. across.

(ii) *Senecio sylvaticus*.—6 weeks: C, 26 mm. across, with 4 leaves; NC, 59 mm. across, with 7-8 leaves. 13 weeks: C, 65 mm. high; NC, 115 mm. high. Both are coming into flower. 24 weeks: C, 175 mm. high, with average of 29 capitula; NC, 210 mm. high, with average of 37 capitula.

(iii) *Centaurea scabiosa*.—7 weeks: C, plants with 2 leaves, the longer 36 mm. long, NC, plants with 4 leaves, the longest 62 mm. long. 9 weeks: C, plants with 4 leaves, the longest 56 mm. long, NC, plants with 6 leaves, the longest 74 mm. long, and considerably broader than the leaves in C. 17 weeks: Equal; plants in both series have average of 10 leaves. 26 weeks: Still equal.

(iv) *Reseda luteola*.—6 weeks: C, 48 mm. across, with 6 leaves; NC, 74 mm. across, with 9 leaves. 9 weeks: C, 95 mm. across; NC, 110 mm. across. 10 weeks: Equal. 13 weeks: plants in C slightly larger than in NC, and with more leaves in healthy condition. 26 weeks: C, 220 mm. across; NC, 170 mm. across, and with fewer leaves.

There remain to be considered those experiments, referred to above, in which the plants on acid (non-calcareous) soil were divided into two groups, one being supplied with a good ration of calcium and the other with hardly any. The results of these experiments were somewhat inconclusive, and they need not be given in detail. It is sufficient to say that in none of the species tested did the substitution of sodium nitrate for calcium nitrate in the nutrient solution have a significantly adverse effect on the growth of the plants. It follows that at any rate a number of calcicole species are able to thrive on a very low calcium supply (which was presumably derived mainly from the Dublin tap-water—not a rich source). It is of interest to note that Marsden-Jones and Turrill (1938) found that *Anthyllis vulneraria*, a moderate calcicole, when grown on soil in which no calcium whatsoever could be detected by the ordinary analytical methods, not only flourished, but yielded on incineration an ash with a remarkably high calcium content.

It does not, of course, follow that some species may not, as was suggested by De Silva (1934), owe their calcicole habit to a high requirement of available calcium in the soil. Some of our own observations suggest that in Ireland this may well be true of *Arum maculatum*, *Tussilago farfara*, and perhaps *Origanum vulgare*. But for the majority of calcicole species some other explanation must be sought.

DISCUSSION.

It would appear from these experiments that a calcareous soil is, in comparison with a mildly acid one, an unfavourable environment for seedlings not only of calcifuge, but also of many calcicole species. This somewhat unexpected conclusion is supported by the general facies of the calcicole flora, whether on chalk down, on esker, or on limestone pavement. All three communities have, in greater or less degree, a characteristic and attractive appearance, which is due to the dwarf habit of most of the plants. As soon as one moves away on to ordinary, neutral grassland one is struck by the rankness of the vegetation. Similarly, if species such as *Carlina vulgaris* or *Erigeron acer* are grown in garden soil, they develop with a startling luxuriance which renders them almost unrecognizable to the field botanist who has seen only the stunted specimens that the esker soil produces. Little is known of the behaviour of calcicole species when grown under controlled conditions with a known pH; but it is significant that Olsen (1921) found that the optimum pH for many indifferent species was well on the acid side of neutrality, and scarcely higher than the optimum for other species that are strongly calcifuge. It would appear that alkalinity is almost universally deleterious.

How then is the calcicole habit to be accounted for? Primarily, it would appear, on a basis of differential susceptibility. Calcicole species are those which, although adversely affected in their early development by calcareous soil, are less seriously affected than are the majority of indifferent or calcifuge species, and are thus given a competitive advantage. It is, of course, well known that competition can play a very important part in determining the habitat of a plant; the classical examples are *Achillea moschata* and *A. atrata* in Central Europe, and *Galium sylvestre* and *G. saxatile* in the British Isles (For the latter, see Tansley, 1917.) In both cases it has been shown that, while under natural conditions the first species of the pair is strictly calcicole and the second strictly calcifuge, either will grow under artificial conditions on either type of soil, if it is protected from competition. The habit of the species is determined under natural conditions by differential survival on each type of soil. Our experiments would seem to suggest that the same factor is at work in determining the calcicole habit of many Irish species; for the fact that these species grow as well, and sometimes better, on acid soils when given the chance indicates that it is only competition from indifferent and calcifuge species that denies them a foothold on these acid soils under natural conditions.

The results of our experiments on calcifuge species present, in themselves, no difficulty; for all prospered, to a greater or less extent, better on non-calcareous than on calcareous soil. But the similarity in the general course of the experiments on *Senecio sylvaticus* and *Digitalis purpurea* to those on calcicole species shows how exceedingly delicate must be the balance of factors involved in successful competitive survival in determining whether the habit be calcicole, calcifuge or indifferent. *Digitalis purpurea*, for example, is, in the broad features of its distribution, one of the most reliable of calcifuge species; and yet the only effect of a calcareous soil on its growth is to produce a temporary check no worse than that suffered by many calcicole species. It is perhaps significant that in its occasional,

sporadic appearances in limestone districts it is usually to be found in small pockets of soil on bare rock (as on the limestone mountains of Co. Sligo) or in earth-filled chinks in walls. One of us has found it in the latter situation near Killarney, in a pocket of soil of which the pH was 7.0. The same wall harboured, in other pockets with the same, or even lower pH, the strongly calcicole species *Geranium lucidum* and *Ceterach officinarum*. This tendency to occur on limestone soils in crannies where there is no continuous carpet of competing vegetation suggests that if the intensity of competition were even slightly reduced the distribution of the species might be much more widespread.

It is necessary, when considering the adverse effect of the calcareous soil on the calcicole species, to realize that it has two very different aspects. The production of pathological symptoms, or of apparently permanent dwarfing, does without doubt take place in some species, and this must represent in all circumstances a very real handicap to the plant in question. But in many other instances the chief effect of the calcareous soil seems to be a delaying one, the seedlings grown on it are, on a given date, smaller and more backward than those grown on the non-calcareous soil, but they represent simply a stage through which the latter have passed some days or weeks before. Now a delay of this kind in early development would clearly be fatal to a seedling that was attempting to compete with others which are not susceptible to the same effect, and were showing normally rapid growth. But if, as would seem probable, all the plants which grow on a highly calcareous soil experience the same effect, then the time factor becomes of no consequence, and the healthier growth ultimately shown on calcareous soil in our experiments by such species as *Geranium lucidum*, *Hypericum perforatum* and *Reseda luteola* may help to determine their calcicole habit.

In conclusion, we must briefly consider the connexion, to which allusion has been made earlier, between the dryness of a soil and its calcium carbonate content. Brenchley (1912) found that certain weeds of arable land that are calcicole in Somerset and Wiltshire are indifferent, or even calcifuge, on the drier soils of E. Bedfordshire; and several species that are markedly calcicole in Ireland are indifferent in S.E. England. But none of the calcicole species dealt with in this paper can be explained purely as dry-soil plants, for their distribution is very different from that of species such as *Hieracium vulgare* or *Linum catharticum* which, though they are most abundant on the calcareous soil of the eskers, are also very common on acid soils wherever the drainage is good enough. But it may be that for some species the two factors—true calcicole habit and preference for dry soils—reinforce each other, and thus restrict the distribution of the species in Ireland more rigidly to calcareous soils than the effect of the chemical factor alone would warrant. *Carlina vulgaris*, for example, is generally speaking a very strict calcicole in Ireland, but it is fairly frequent on steep, sandy and gravelly banks in Glendalough and Glenmalur which are quite free from calcium carbonate (Praeger, 1933, and personal observations). It would seem probable that this species is really a dry-soil plant and also a moderate calcicole; but that the scarcity of well-drained non-calcareous soils in Ireland transforms it effectively into a strict calcicole.

SUMMARY.

Twenty-two species of plants, of which fifteen are calcicole in their Irish distribution, and seven calcifuge, have been grown from seed under identical conditions in two artificial soils, one containing calcium carbonate, the other free from it.

In general the calcareous soil was found to have an adverse influence on both calcifuge and calcicole species. Of the calcicoles, *Blackstonia perfoliata* failed completely on the acid soil, *Origanum vulgare* and *Poterium sanguisorba* showed similar behaviour throughout on the two soils, and the remaining twelve were at some period of their growth (usually in the seedling stage) adversely affected by the calcareous soil. In nine species this initial handicap was later made up, and the plants on the calcareous soil eventually made as good (in six species, slightly better) growth as those on the acid soil. Of the calcifuge species, *Radiola linoides* and *Juncus squarrosus* failed completely on the calcareous soil, the others were adversely affected by it, on the whole more severely than were the calcicole species, but there was, in their cultural behaviour, no clear-cut division between the two groups.

It is suggested that the calcicole habit must be largely determined by differential survival in an environment that is universally unfavourable; but that the balance between environmental and competitive factors must be exceedingly delicate in determining whether the habit be calcicole, calcifuge, or indifferent. The significance of the dryness of most calcareous soils is discussed.

No evidence was obtained to suggest that, in any of the species selected for experiment, the concentration of available calcium in the soil is *per se* a major factor in influencing distribution.

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No. 4.

THE ANGULAR DISTRIBUTION OF SUBMARINE DAYLIGHT IN DEEP WATER.

By

H H POOLE.

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A NUMBER of workers have studied the angular distribution of daylight beneath the surface of the sea or of lakes. In particular, Johnson and Liljequist (4) have made many measurements beneath the surface of Swedish fjords, using a photometer sensitive only to a narrow cone of rays, and adjustable in direction so as to enable polar curves of the illumination to be plotted for any desired vertical plane. Most of their curves refer to sunlit conditions with a smooth water surface. In general they found that the increased extinction of oblique rays caused the obliquity of the maximum radius vector of their curves to decrease with increase of depth, the curves usually becoming relatively less elongated at the same time.

There does not appear however to be any decisive evidence in favour of a decrease in the vertical extinction coefficient such as would accompany a decrease of average obliquity with increase of depth. In fact, for monochromatic light in homogeneous water, the coefficient appears to vary little with change of depth in unsheltered waters where the obtaining of such polar curves would be very difficult. Lack of homogeneity will of course affect the coefficient one way or the other, and the use of too wide a spectral band will cause it to decrease owing to the filtering-out of the less penetrating wave-lengths. Moreover, in measurements made by Atkins off Plymouth (1) no correlation has ever been found between the vertical extinction coefficient at depths exceeding 20 metres and either the degree of roughness of the surface or the nature of the daylight, whether entirely diffuse or low or high sunlight.

This suggests that the average obliquity tends to a constant value governed by a balance between the opposing effects of absorption and scattering, one decreasing and the other increasing the obliquity with increase of depth. Some evidence in support of this view is furnished by measurements of the ratio of the horizontal to the vertical illumination made by Atkins in 1938-1939 (2). These failed to show any regular change with depth in the ratio horizontal/vertical.

Johnson and Liljequist's results were mostly obtained nearer the surface, where the distribution was evidently largely governed by that of the daylight above, but even in their case it is not obvious that the broadening of their curves with increase of depth does not off-set the reduction in obliquity of their major axes, so that the average obliquity may be little affected.

EQUILIBRIUM DISTRIBUTION ON THE ASSUMPTION OF SPHERICAL SCATTERING.

It may be of interest to consider the distribution to which we should expect the balance between absorption and scattering to lead, and to see how far it can be reconciled with the experimental results. The problem of the contribution made by scattered light has been theoretically considered by Le Grand (6), and by Whitney (8). These authors deal chiefly with moderate depths, at which the scattered light forms so small a part of the whole that multiple scattering may be neglected. They both assume as an approximation that the light scattered by the diffusing material in any small element of volume is equally distributed in all directions, regardless of the direction of the incident light.

This simplifying assumption is also made below. It would be true if the scattering were due solely to reflection by polished spherical particles large compared with the wave length, but, in making it for sea water in order to make the mathematics manageable, we must remember that our only justification is the large variation likely to occur in the sizes and natures of the particles in suspension, some large compared with the wave length, causing a preponderance of backward irregular reflection if opaque or of forward transmission if transparent, others comparable in size with the wave length giving a distribution varying with their diameters, the intensity being a minimum perpendicular to the incident light for very small particles. Le Grand (*loc. cit.* p. 404 *et seq.*) gives an account of these effects, and shows that in sea water scattering by the molecules is generally small compared with that by particles in suspension.

Let us suppose that a parallel beam of monochromatic radiation of flux F , in passing through a thin perpendicular layer of sea water of thickness dx , loses by absorption and scattering $cFdx$, of which a fraction $scFdx$ is due to scattering in all directions, the remainder being due to absorption. We may call s , i.e. the fraction of the total extinction that is due to scattering, the 'scattering fraction.'

Suppose that direct sunlight falls on a smooth water surface at such an angle that its refracted path in the water makes an angle α with the vertical. Let the intensity of illumination on a surface perpendicular to the beam just below the surface be I_0 lux (i.e. lumens per sq. metre). Then at a depth x the intensity of the direct sunlight will be $I_0 e^{-cx \sec \alpha}$, but, even if we suppose that there is no diffuse sky light, the vertical intensity recorded by a horizontal photometer will, in general, not fall off according to the exponential law, as the relative importance of the vertical component of the scattered light will increase with the depth. This effect is considered quantitatively by Le Grand, who, however, deals chiefly with the upper layers where the contribution of the scattered light is so small that doubly scattered light may be neglected. In what follows we are concerned with the lower layers in which equilibrium angular distribution has been reached. It is evident that this cannot occur until all the light has been scattered at least once.

If the water is deep enough we may, for the levels in which we are interested, neglect reflection off the bottom. The illuminations at two points P and Q will have the same angular distribution and only differ by a factor depending on their difference in level. If that at the upper point P is multiplied by any number that at Q will be multiplied by the same number, since the latter is due solely to the illumination

passing downward through the *P* level. Hence it is easily seen that once equilibrium has been reached the vertical extinction of the diffuse light follows an exponential law. This would generally not be true near the bottom, where reflected light would cause a variation in the angular distribution with depth.

Let us adopt the following notation, using the metre, the lux, and the lumen, as units of length, intensity of illumination, and flux

c = Coefficient of extinction (including absorption and scattering) for a parallel beam.

s = Scattering fraction, i.e. fraction of the total light removed from a beam that is due to scattering (the rest of the loss being due to absorption). It is assumed that this scattering is equally distributed in all directions.

μ = Coefficient of vertical extinction attained at depths at which the angular distribution has reached equilibrium.

$k = \mu/c$.

J = Total flux per sq. metre of diametral cross section received by a spherical photometer from the lower hemisphere. The diametral cross section is evidently $1/4$ of the total area of the photometer.

K = Total flux similarly received from the upper hemisphere.

$L = J + K$ = total or integral flux per sq. m. at the point considered, i.e. the illumination effective in causing photo-synthesis.

U = Flux per sq. m. received on a horizontal plane photometer facing downwards (i.e. upward vertical illumination in lux).

V = Flux per sq. m. received on a similar photometer facing upwards (i.e. downward vertical illumination in lux, as commonly measured).

H = Flux per sq. m. of vertical diametral section received by a cylindrical photometer with its axis vertical. The diametral section is evidently $1/\pi$ of the curved vertical surface of the photometer.

B = Brightness of field in lux per steradian as viewed from the photometer. The brightness in lamberts will be $\pi B \times 10^{-4}$.

In every case the photometer surface is assumed to absorb in accordance with the cosine law.

Since equilibrium has been attained and the angular distribution is constant the ratios $J : K : L : U : V$ are independent of the depth; each of these quantities decreasing exponentially with a vertical extinction coefficient μ , i.e. kc .

We shall in future consider these quantities as referring to the point *O*, the position of the photometer.

Let *PQ* in Fig. 1 be an element of volume in the form of a thin slab of the medium of area *A* and thickness *dx* exposed to parallel illumination of intensity *I*.

lux (as measured on a perpendicular photometer) falling on the slab at an angle of obliquity α . The element will scatter $IA \cos \alpha \sec \alpha dx$ i.e. $Iscdv$ lumens, where dv is its volume. As this is independent of α the flux scattered by a volume element exposed to total illumination L from all directions will be $Lscdv$. We assume that this scattering takes place equally in all directions, as indicated in the figure, so that the volume element acts as a source of intensity $Lscdv/4\pi$ candle power

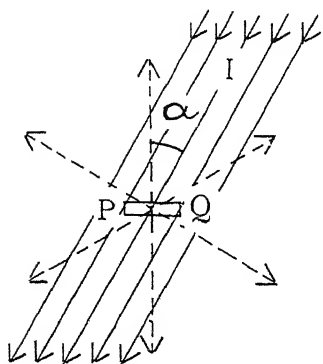


Fig. 1.

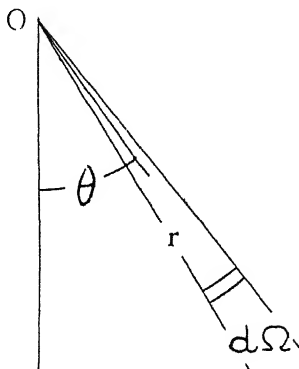


Fig. 2.

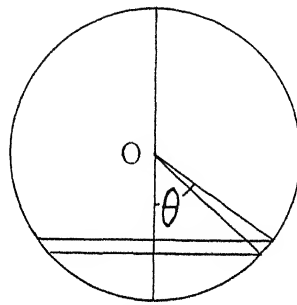


Fig. 3.

Consider the brightness of field B in a direction making an angle θ with the downward-drawn vertical through O (see Fig. 2). Evidently, considering the illumination received at O from a small solid angle $\delta\Omega$ in the given direction, we have

$$B\delta\Omega = \int_0^\infty \frac{scLe^{-\mu r \cos \theta}}{4\pi} \cdot r^2 \delta\Omega \cdot \frac{e^{-c r}}{r^2} \cdot dr$$

$$\text{so } B = \frac{scL}{4\pi} \int_0^\infty \frac{e^{-(1+k \cos \theta)cr}}{e} dr$$

$$\text{or } B = \frac{sL}{4\pi (1+k \cos \theta)} \quad (1)$$

where L is now the total illumination at O , provided that $1+k \cos \theta$ is always positive, i.e. that $k < 1$. We shall find that this condition is satisfied for all possible values of s , but that if the latter is small k is very close to unity. This would imply that equilibrium is only reached at great depths.

It is interesting to note that on the assumption of spherical scattering the vertical extinction coefficient μ is always less than the extinction coefficient for a parallel beam c , the contribution made by the scattered light more than offsetting the increased extinction due to obliquity.

Equation (1) shows that the equilibrium polar surface of brightness of field is an ellipsoid of eccentricity k , O being the lower focus. As k would generally be not much less than unity the ellipsoid would be much elongated.

Integrating over a sphere as indicated in vertical section in Fig. 3 we find

$$\begin{aligned}
 L &= \int_0^\pi B \, 2\pi \sin \theta \, d\theta = \frac{sL}{2} \int_0^\pi \frac{\sin \theta \, d\theta}{1+k \cos \theta} \\
 &= \frac{sL}{2k} \log \frac{1+k}{1-k} \\
 \therefore s &= \frac{2k}{\log \frac{1+k}{1-k}} \quad (2)
 \end{aligned}$$

The logs being to the base e .

This shows the relation between s and k . Evidently if $s=0$ (no scattering) $k=1$, equilibrium only being reached at an infinite depth where absorption has rendered the light purely vertical. As s increases k decreases, thus justifying our assumption that $k < 1$. If s is near unity (absorption small) k is small, the conditions approximating to those obtaining in a thick cloud. Values of k for different values of s are plotted in Fig. 4.

Dividing L into its lower and upper parts we have

$$J = \frac{sL}{2} \int_0^{\pi/2} \frac{\sin \theta}{1+k \cos \theta} = \frac{sL}{2k} \log (1+k) \quad (3)$$

$$\text{and } K = \frac{sL}{2} \int_{\pi/2}^\pi \frac{\sin \theta}{1+k \cos \theta} = \frac{sL}{2k} \log \frac{1}{1-k} \quad (4)$$

We are generally more interested, however, in U and V , the illuminations recorded by down- and upturned plane photometers

$$\text{We have } U = \int_0^{\pi/2} B \, 2\pi \sin \theta \cos \theta \, d\theta = \frac{sL}{2} \int_0^{\pi/2} \frac{\sin \theta \cos \theta \, d\theta}{1+k \cos \theta}$$

$$\text{Hence (see Appendix A) } U = \frac{sL}{2} \frac{k - \log (1+k)}{k^2} \quad (5)$$

$$\text{Similarly } V = - \int_{\pi/2}^\pi B \, 2\pi \sin \theta \cos \theta \, d\theta$$

$$\text{so } V = \frac{sL}{2} \frac{\log \frac{1}{1-k} - k}{k^2} \quad (6)$$

To measure the horizontal component of the illumination we would need a vertical cylindrical photometer surface exposing the same effective area to light from all azimuths.

$$\text{Evidently } H = \int_0^\pi B \, 2\pi \sin^2 \theta \, d\theta = \frac{sL}{2} \int_0^\pi \frac{\sin^2 \theta \, d\theta}{1+k \cos \theta}$$

Hence (see Appendix B)
$$H = \frac{\pi s L (1 - \sqrt{1 - k^2})}{2k^2} \quad (7)$$

From (5) and (6) we get
$$\frac{U}{V} = \frac{k - \log (1+k)}{\log \frac{1}{1-k} - k} \quad (8)$$

from (7) and (6)
$$\frac{H}{V} = \frac{\pi (1 - \sqrt{1 - k^2})}{\log \frac{1}{1-k} - k} \quad (9)$$

(3), (4), (2) and (6) give
$$\frac{J}{V} = \frac{k \log (1+k)}{\log \frac{1}{1-k} - k} \quad (10)$$

$$\frac{K}{V} = \frac{k \log \frac{1}{1-k}}{\log \frac{1}{1-k} - k} \quad (11)$$

and
$$\frac{L}{V} = \frac{k \log \frac{1+k}{1-k}}{\log \frac{1}{1-k} - k} = \frac{2k^2}{s(\log \frac{1}{1-k} - k)} \quad (12)$$

(3) and (5) give
$$\frac{J}{U} = \frac{k \log (1+k)}{k - \log (1+k)} \quad (13)$$

Several workers have made measurements of U/V , which is generally a few per cent. A few measurements have been made of H/V , those by Atkins in 1938-9 being around 80 per cent. L/V is of interest as showing the ratio of the photosynthetic activity of the light to the vertical illumination as usually recorded. K/V measures the mean obliquity of the downward-travelling light, while J/U gives the corresponding obliquity for that travelling upwards.

For values of k not exceeding 0.99 (for which $s=0.375$) it is convenient to assume values for k and hence from (2) and (8) to (13) find the corresponding values of s and the ratios U/V etc., as plotted in Fig. 4. For values of k very close to unity it is more convenient to use approximate formulae, taking assumed values of s . In this case

(2) becomes
$$\log \frac{1}{1-k} = \frac{2k}{s} - \log (1+k) = \frac{2}{s} - 0.693 \text{ nearly}$$

(8) "
$$\frac{U}{V} = \frac{0.307 s}{2 - 1.693 s}$$

(9) "
$$\frac{H}{V} = \frac{\pi s}{2 - 1.693 s}$$

(10) "
$$\frac{J}{V} = \frac{0.693 s}{2 - 1.693 s}$$

$$(11) \text{ becomes } \frac{K}{V} = \frac{2 - 0.693 s}{2 - 1.693 s}$$

$$(12) \quad \text{,,} \quad \frac{L}{V} = \frac{2}{2 - 1.693 s}$$

$$(13) \quad \text{,,} \quad \frac{J}{U} = \frac{0.693}{0.307} = 2.26$$

$$\text{Also } \frac{H}{U} = \frac{\pi}{0.307} = 10.2$$

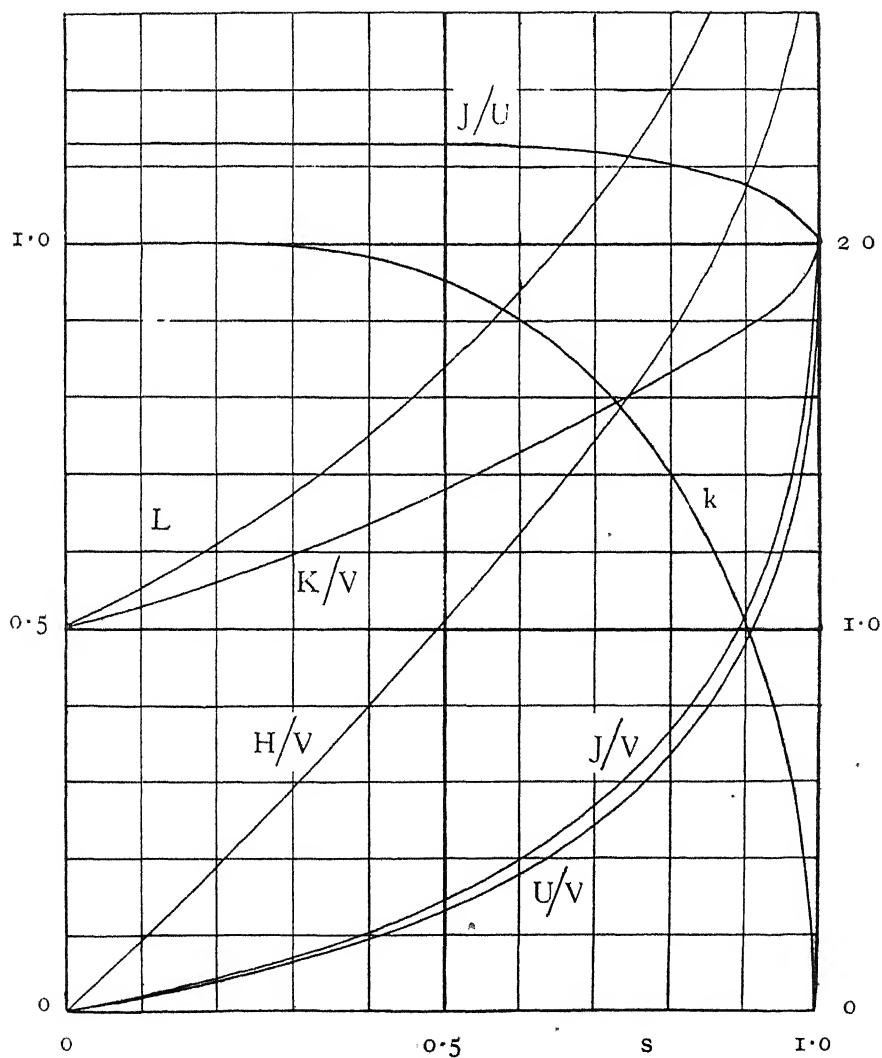


Fig. 4.

These approximations may be made for values of s up to 0.3, which corresponds to $k=0.9975$. It is worth noting that for these small values of s the average obliquity of the upward-scattered light is almost constant, and may be represented by $\sec^{-1} 2.26$, i.e. nearly 64° , while the horizontal light recorded by a cylindrical photometer is about 10 times the upward light. We shall find later that this theoretical result for the ratio H/U does not seem to be borne out by experience.

The ratios found from equations (2) and (8) to (13) are plotted against s in Fig. 4, k and U/V in accordance with the scale on the left, the remainder on the half scale shown on the right.

A number of measurements of U/V have been made, notably by Utterback (6) in the Pacific, Clarke (3) in the Western Atlantic, and Atkins (1) in the English Channel. A few measurements of H/V have also been made, of which those made by Atkins are considered here. From the experimental values of U/V or from those of H/V we can read from the figure the corresponding values of s and of the other ratios on the assumption of equilibrium distribution and spherical scattering, and thus if U/V and H/V are measured simultaneously we can test the assumptions made.

We see from the figure that as s rises from 0 to 1, L/V rises from 1 (purely vertical light) to 4 (perfectly diffuse light), K/V , the mean secant of the obliquity of the downward light, rises from 1 to 2, and J, U , the mean secant of the obliquity of the upward light falls from an almost stationary value 2.26 to 2.00. It may be noted that the upward light is always more oblique than the downward, so that the ratio U/V under-rates its photosynthetic importance. The fall of k , i.e. μ/c , with increasing s in spite of the increasing obliquity shows the great importance of scattered light in reducing the vertical extinction.

COMPARISON WITH EXPERIMENTAL RESULTS

We may compare the above results with some measurements made with a cubical photometer by Atkins off Plymouth in 1938-39 (2). These gave simultaneous values of U/V and H/V . Unfortunately the few series were only of a preliminary nature, further work being prevented by the outbreak of war.

The results are shown in the table, in which the series are arranged in order of decreasing values of U/V , which only makes one slight deviation from decreasing order of μ . Percentages are used for convenience in printing.

TABLE.

| Series | μ % | U/V % | $U/\mu V$ % | s % | H/V % | s^2 % | s^2/s |
|-----------|------------|------------|----------------|----------|------------|------------|---------|
| C 1 | 20.0 | 5.50 | 27.5 | 27.5 | — | — | — |
| C 5 | 13.5 | 3.45 | 25.6 | 18.9 | 84 | 42 | 2.22 |
| C 4 | 11.5 | 3.17 | 27.6 | 17.6 | 80 | 40 | 2.28 |
| C 3 | 11.0 | 3.02 | 27.5 | 16.9 | 73 | 37 | 2.19 |
| C 6 | 11.2 | 2.98 | 26.6 | 16.7 | 81 | 40 | 2.39 |
| C 7 | 7.6 | 2.10 | 27.6 | 12.3 | 80 | 40 | 3.25 |

C 1 and C 6 .. Low Sun Strong Wind.

C 7 High Sun. Strong Wind.

C 5, C 4 and C 3 .. Overcast. Very Light Wind.

It has been pointed out by J. G. Moore (private communication) that, as shown in the 4th column, $U/\mu V$ is almost constant. There does not, however, appear to be any evident theoretical justification for this relation beyond the qualitative one that as s is small in pure water it will naturally tend to increase with the amount of matter in suspension, so that both μ and U/V will increase together. In finding the values of s given in the table use was made of the approximate form of (8) which is applicable to the low values concerned, and gives more accurate results than could be read from the figure.

No values are shown for H/V for Series C1, which was made with the ship anchored inside Plymouth breakwater with strong sunlight, as rotation of the photometer rendered the interpretation of the results difficult. The mean values for depths of 10 m. and over for the other series are shown, and the corresponding values, s^1 , of the scattering fraction, as read from the curve. On our assumptions s^1/s should equal unity, instead of being around two or three.

No very great weight can be attached to these few results which were obtained under conditions which were not ideal, especially as trouble was experienced with the photo-cells, whose sensitivity fell considerably in the course of a few months. Moreover, in Series C6 and C7 the combination of bright sun, strong wind, and rough sea, rendered the determination of H/V difficult, and the interpretation of the results uncertain. The sun was low in C6 and high in C7, but in both cases the rough surface with breaking waves must have rendered the average obliquity comparatively large just below the surface, and there was no obvious difference in the recorded mean values of H/V . It was evident in both series that even at 40 m. equilibrium distribution was far from being attained, as at that depth the illumination on a vertical surface perpendicular to the sun's azimuth exceeded the mean for other azimuths by about 20 per cent. in C6, and as much as 48 per cent. in the clearer water of C7.

They are, however, the only simultaneous measurements of U , V , and H with which I am acquainted. Earlier comparisons by various workers in which the photometer was successively suspended at different angles can hardly be regarded as very satisfactory, owing to possible changes in the conditions in the interim, or to possible differences in depth owing to variations in the rate of drift of the ship. Utterback's early simultaneous measurements of U and V (7) were made with a photometer without diffusing glass, and sensitive only to a comparatively narrow angle. They naturally gave smaller values for U/V owing to the greater average obliquity of the upward light.

The results, for what they are worth, suggest very strongly that the ratio H/U is two or three times as great as would be expected on the assumption of uniform scattering. Even though equilibrium had not been attained the absence of any tendency of either U/V or H/V to change regularly with depth seems to imply that their values were close to the equilibrium ones. We shall see below that the values found for H/V are also close to those to be expected just below a calm surface illuminated by a uniform overcast sky.

The greater part of H is supplied by zones somewhat above the horizontal, for which the average total scattering (much of it multiple) is through angles from 45° to 90° , whereas U is mainly due to light which has been scattered through

angles from 135° to 180° . It would appear, therefore, that single scattering through comparatively small angles is considerably greater than that through angle exceeding 90° .

This is the exact opposite of the diffuse reflection that would be produced by unpolished particles large compared with the wave length, nor does it agree with the true scattering by particles very small compared with the wave length. The latter is just twice as large for angles near 0° or 180° as for 90° , the distribution being symmetrical about the 90° plane.

The effect might be produced by refraction through comparatively large transparent particles, or by true scattering by particles comparable with the wave length (see Le Grand *loc. cit.*, p. 407).

It is evident from the figure that the values of U/V recorded give values of k only very slightly less than unity, while for the values of H/V k is about 0.985. This would imply that angular equilibrium would only be attained at great depths if the scattering were uniform (see derivation of equation 1). As we have seen, however, there is evidence that the scattering through small angles exceeds that through large, which would hasten the attainment of equilibrium. We could not, of course, expect the polar surface to be ellipsoidal.

Although it would appear that, owing to the nature of the scattering particles, the theoretical results given above do not apply quantitatively to sea water, it would appear to be reasonable to draw some qualitative conclusions from them. Thus from equation (8) we see that U/V is a function of k , and hence of s only, and not an explicit function of μ . This should still be true even if the scattering is not uniform if we interpret s as a measure of light scattered through large angles. The colour of the upward scattered daylight therefore depends only on the variation of s with wave length. In ocean water the total extinction is a minimum in the blue in spite of the fact that, in the comparative rarity of particles in suspension, molecular scattering, which is inversely proportional to the fourth power of the wave length, must add appreciably to the total scattering in that spectral region. The decrease in extinction is due to reduced absorption by the water, which is most transparent in the blue. Increased scattering and reduced absorption combine to raise s and hence U/V . As the molecular scattering increases with reduction in wave length we should expect the maximum value of s to occur deeper in the blue than the region of maximum transmission. Thus ocean water looks a deeper blue than the colour for which the transmission is greatest. Further inshore the maximum transmission shifts towards the green. This can hardly be entirely due to increased scattering of the short waves by the greater number of particles in suspension, as if so the water would maintain its deep blue tint, whereas the maximum value of U/V is now in the green. It seems to be more probably due to absorption of the blue light, either by particles of a yellowish or greenish colour, or by a dye or stain in solution, as suggested by Kalle (5), which would, however, tend to reduce U/V . In turbid water almost all the extinction is due to the particles (except possibly in the red, where the water absorbs very strongly), and the colour is determined by that of the particles. If for them s is independent of the wave length the water will appear grey. See Le Grand *loc. cit.*, p. 429, and bibliography of earlier authors.

ANGULAR DISTRIBUTION JUST BELOW A CALM SURFACE.

It may be of interest to compare the values actually found for H/V with that to be expected just below a smooth water surface illuminated by a uniform overcast sky. We shall neglect the loss of light by reflection at the surface. This simplification will exaggerate the importance of light whose obliquity in the water is only slightly less than the critical angle 48.6° . The approximation will tend to allow for the effects of a slight ruffling of the surface, such as will almost always occur at sea under even the calmest conditions.

It is easy to show that the light just below the surface will be limited to a vertical cone of semi-angle γ equal to the critical angle, the brightness of field within the cone being n^2 times that of the sky, where n is the refractive index of the water. The brightness B is thus uniform over the cone.

$$\text{Evidently } V = 2\pi B \int_0^\gamma \sin \theta \cos \theta \, d\theta$$

$$= \frac{\pi B}{2} (1 - \cos 2\gamma)$$

$$\text{and } H = 2\pi B \int_0^\gamma \sin^2 \theta \, d\theta = \pi B \left(\gamma - \frac{\sin 2\gamma}{2} \right)$$

$$\therefore H/V = \frac{2\gamma - \sin 2\gamma}{1 - \cos 2\gamma}$$

Taking n as 1.33 we find $\gamma = 48.6^\circ$ and $H/V = 0.626$.

This, however, takes no account of scattered light from the lower hemisphere. As U/V is never more than a few per cent, and as internal reflection from the surface is also small for angles within the critical angle, we may neglect the contribution of U to V . The contribution to H is, however, appreciable, as it includes the horizontal components, not only of the entire lower hemisphere, but also of the total reflection by the surface of that part of it lying beyond the critical angle. We can only make an approximate allowance for this contribution in view of the deviation between the theoretical and the experimental results for H/U . If, however, we assume as a value for s the mean of the results found from U/V in Series C 3, C 4, and C 5, i.e. 0.178, we shall at least get a fair value for the upward component of the lower hemisphere, and shall probably not grossly underestimate its horizontal component, since the greater part of the excessively high value of H/U found by measurement probably comes chiefly from the upper hemisphere (which is of course absent, as far as scattered light is concerned, just below the surface).

This value of s makes k very close to unity, the approximate form of (2) showing that $1 - k = 2e^{-2/s}$, which amounts to about 2×10^{-5} . This means that in

the integral given in Appendix B the term involving $\sqrt{1-k}$ only becomes appreciable when θ approaches π making $\tan \frac{\theta}{2}$ large. Thus it becomes a very simple matter to find the value of the integral for the whole lower hemisphere and add, for the reflected light, the value for the part outside the critical angle. When this is done we find that we should add 0.110 to the value of H/V to allow for the total effect of the lower hemisphere. This raises the theoretical value of H/V just below a calm surface to 0.726, an estimate which is probably slightly low.

We see then that the values actually found for H/V are not very different from those to be expected just below a calm surface illuminated by a uniform sky. The fact that no regular variation with depth was found in these values seems to indicate that they also approximate to the equilibrium value reached in deep water. The latter therefore seems, for the conditions under which these measurements were made, not to differ greatly from the value to be expected just below the surface. Further measurements are however very desirable.

SUMMARY.

(1) Experimental results show little if any regular variation with depth in the vertical extinction coefficient or in the ratio of the horizontal to the vertical illumination, if the measurements are made for approximately monochromatic light in homogeneous water. The extinction coefficient seems to be only slightly affected by the state of the water surface or by the angular distribution of the daylight.

(2) This has led to the suggestion by more than one worker that the angular distribution, and hence the average obliquity, tend to be determined by a balance between the opposing effects of absorption and scattering, the first decreasing the mean obliquity and the second increasing it.

(3) An attempt is here made to present the theory of such an equilibrium distribution on the assumption that the scattering of a beam of light by the particles in suspension takes place equally in all directions. The polar surface of field brightness so found is an ellipsoid of revolution about a vertical axis, the point of observation being the lower focus.

(4) It is shown that, on this hypothesis, the equilibrium value of the vertical extinction coefficient is always less than the extinction coefficient (including all scattering) for a parallel beam. Where only a comparatively small part of the extinction is due to scattering the difference between the two coefficients is very small. This would mean that equilibrium would only be approached very slowly.

(5) Comparison of the theory with a few simultaneous measurements of the downward, upward, and horizontal illuminations made off Plymouth in 1938-1939 shows lack of agreement, the results apparently showing a considerable excess of scattering through angles less than 90° . This seems to indicate that the scattering is mainly due either to transparent particles large compared with the wave length or to particles comparable in size with the wave length.

(6) Although this discrepancy prevents the theory from being applied quantitatively to the conditions under which the measurements were made it would seem likely that some qualitative deductions may be drawn from it. Thus it shows that the ratio of the upward to the downward illumination (i.e. the albedo of the underlying water) depends only on the relative importance of scattering and absorption in producing extinction, and not directly on the total value of the extinction coefficient. This explains how the variation of the albedo with wave length and hence the colour of the water, may be expected to depend on the nature of the water.

(7) Measurements made at various depths below a comparatively calm surface with a cloudy sky gave a value for the ratio of the horizontal to the vertical illumination close to that to be expected just below the surface. The approximate constancy of this ratio as the depth increased indicated that it was also close to the equilibrium value reached in deep water. Further measurements are, however, very desirable.

APPENDIX.

A.

$$\begin{aligned} \int \frac{\sin \theta \cos \theta d\theta}{1+k \cos \theta} &= -\frac{1}{k} \left[\cos \theta \log (1+k \cos \theta) + \int \sin \theta \log (1+k \cos \theta) d\theta \right] \\ &= -\frac{1}{k} \left[\cos \theta \log (1+k \cos \theta) - \frac{1+k \cos \theta}{k} \left\{ \log (1+k \cos \theta) - 1 \right\} \right] \\ &= \frac{1}{k^2} \left[\log (1+k \cos \theta) - (1+k \cos \theta) \right] \end{aligned}$$

$$\text{Hence } \int_0^{\pi/2} \frac{\sin \theta \cos \theta d\theta}{1+k \cos \theta} = \frac{k - \log (1+k)}{k^2}$$

$$\text{and } -\int_{\pi/2}^{\pi} \frac{\sin \theta \cos \theta d\theta}{1+k \cos \theta} = -\frac{k + \log (1-k)}{k^2} = \frac{\log \frac{1}{1-k} - k}{k^2}$$

B.

$$\begin{aligned} \int \frac{\sin^2 \theta d\theta}{1+k \cos \theta} &= \frac{1}{k^2} \int \frac{(1-k^2 \cos^2 \theta) - 1 + k^2}{1+k \cos \theta} d\theta \\ &= \frac{1}{k^2} \int (1-k \cos \theta) d\theta - \frac{1-k^2}{k^2} \int \frac{d\theta}{1+k \cos \theta} \\ &= \frac{\theta - k \sin \theta}{k^2} - \frac{1-k^2}{k^2} \int \frac{d\theta}{1+k \cos \theta} \end{aligned}$$

$$\begin{aligned}\text{Now } \int \frac{d\theta}{1+k \cos \theta} &= \int \frac{d\theta}{(1+k) \cos^2 \frac{\theta}{2} + (1-k) \sin^2 \frac{\theta}{2}} \\ &= \frac{1}{1+k} \int \frac{\sec^2 \frac{\theta}{2} d\theta}{1 + \frac{1-k}{1+k} \tan^2 \frac{\theta}{2}}\end{aligned}$$

$$\text{Let } \sqrt{\frac{1-k}{1+k}} \tan \frac{\theta}{2} = \tan \varphi$$

$$\text{so } \sqrt{\frac{1-k}{1+k}} \sec^2 \frac{\theta}{2} \frac{d\theta}{2} = \sec^2 \varphi d\varphi$$

$$\begin{aligned}\text{Then } \int \frac{d\theta}{1+k \cos \theta} &= \frac{2}{\sqrt{1-k^2}} \int \frac{\sec^2 \varphi d\varphi}{1+\tan^2 \varphi} \\ &= \frac{2\varphi}{\sqrt{1-k^2}} = \frac{2}{\sqrt{1-k^2}} \tan^{-1} \left\{ \sqrt{\frac{1-k}{1+k}} \tan \frac{\theta}{2} \right\}\end{aligned}$$

$$\text{Hence } \int_0^\theta \frac{\sin^2 \theta d\theta}{1+k \cos \theta} = \frac{1}{k^2} \left[\theta - k \sin \theta - 2\sqrt{1-k^2} \tan^{-1} \left\{ \sqrt{\frac{1-k}{1+k}} \tan \frac{\theta}{2} \right\} \right]$$

The integral vanishing at the lower limit

$$\text{So } \int_0^\pi \frac{\sin^2 \theta d\theta}{1+k \cos \theta} = \frac{\pi (1-\sqrt{1-k^2})}{k^2}$$

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No 5.

DEVELOPMENTAL LINES IN POLLINATION MECHANISMS IN THE CONIFERALES

By

JOSEPH DOYLE

(UNIVERSITY COLLEGE, DUBLIN)

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POLLINATION, and its relation to ovular structure, have been described in a series of recent papers by Doyle and O'Leary (1935 a, b, c) and Doyle and Kane (1943). These papers have special reference to the Pinaceae, and, with the exception of two genera, the phenomena are now fairly clear in that family. Although detailed observations have still to be made in many genera of other families, yet, from the information already at hand about these, it seems possible and timely to offer suggestions concerning at least some of the lines along which modifications in pollination mechanisms may have taken place in the Coniferales as a whole, and to indicate also where information is particularly lacking. The tracing of these lines has been made feasible by recent extensions of our knowledge of Carboniferous and Permian conifers by Walton (1928) and particularly by Florin (1938-9, 1939).

It is to be emphasized that this paper is not concerned with problems such as the immediate phyletic relationships of individual living and fossil genera, but solely with the relationships between types of pollination mechanisms as far as these are known.

THE PALAEOZOIC TYPES.

The sections at present available of Florin's monograph (1938-9) on Palaeozoic conifers provide details of the structure of the gynostrobilus, ovule, and pollen grain, of several species of *Lebachia* (the old genus *Walchia* in part). Other genera are considered in sections not available, but general features have been summarized in a preliminary note (Florin 1939). These ancient members of the Coniferales appear to be related to the better known Cordaitales, although not descended from them, the two groups representing two lines of descent from some still earlier phylum. It is thus of interest that the *Lebachia* species had a grain, and presumably also a pollination mechanism, similar to that of the Cordaitales, and that the grain definitely differed from that of the other large Palaeozoic order, the Pteridospermae (Florin 1937). It is then here assumed that some such type as that described for *Lebachia* is basal and primitive in the conifers.

The cone of *L. pumiformis* shows a close series of spirally arranged bracts in the axils of most of which stands a fertile dwarf shoot (Fig. 1 A). This shoot has a number of sterile appendages, clearly reduced telomes, one of which carries a single

erect ovule. The extine of the pollen, Fig 1 B, expanded into a large balloon-like air sac, *a*, covering the whole of the grain except for a small area at that part called by Florin the distal pole, *p*, corresponding in position to the furrow (cf. Wodehouse 1935) in modern grains. The Pteridospermae did not possess a balloon air sac, which was however characteristic of the Cordaitales (Florin 1936). Further, since the grains passed through the micropylar canal to lodge in the pollen chamber of the nucellus, it would seem that fluid must have been exuded from the micropyle, some type of pollination drop in which the air-borne grains were caught. The details of subsequent phenomena must naturally always remain obscure, but the structure

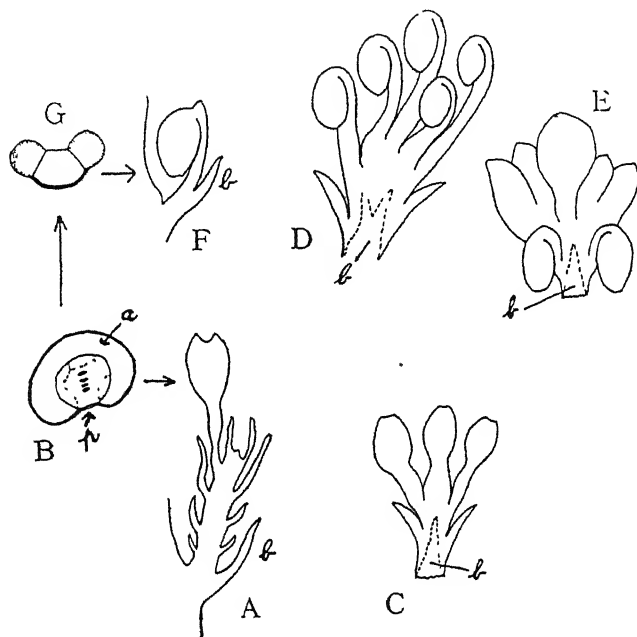


FIG 1. Ovuliferous structures in Palaeozoic conifers (diagrammatic after Florin and Walton). A. *Lebachia piniformis*. B. Pollen of same, *a*, balloon air-sac; *p*, distal pole. C. *Ernestrodendron filiciforme*. D. *Walchia germanica*. E. *Pseudovoltzia Liebeana*. F. *Ullmannia Bronni*. G. Pollen of same. In each *b*=cone bract.

of the grains suggests that the air-sac round it was a flotation device of such a nature that the grain would tend to float with its distal pole, *p*, the probable position of germination, underneath. The slow or rapid reabsorption of the pollination fluid would cause the grain to be drawn into the pollen chamber orientated, at least roughly, with its germinating or fission area below. Florin's (1938-9, Heft 1) statement that the cones of *L. piniformis* are "stets ± aufrecht gestellt" supports this suggestion.

It may be taken as certain that some exudation of pollination fluid was a feature, not only of these early conifers, but of all the primitive gymnosperms, Cordaitales and Pteridospermae alike. It is still a feature of the living Cycadales, the Gnetales, and *Ginkgo*. It is also shown in all families of the living Coniferales.

except the Araucariaceae, in which a specialized type of mechanism has been developed. An exudation of pollination fluid from the ovule is thus taken as basal in the Coniferales.

Many other types of these Upper Carboniferous and Permian conifers are known. Certain simple features of some of them are illustrated in Fig. 1, modified very diagrammatically from Florin (1939) and Walton (1928). All of them showed a cone, as in *Lebachia*, with a series of spirally arranged bracts, each bract carrying in its axil a special reproductive dwarf shoot. In each case, in Fig. 1, a single such shoot is outlined, either isolated, as in C, D, E, with the abaxial bract, *b*, dotted, or in relation to the bract and main cone axis, as in B, G. *Lebachia* with its single terminal ovule (1 A) has already been referred to. In other forms, notably species of *Walchiostrobus*, several of the telomic appendages on the reproductive shoot carried erect ovules, the shoot and its appendages tending to become broadened and flattened. This tendency is well expressed in *Ernestiodendron filiciforme* Florin, Fig. 1 C, in which the axillary fertile shoot became itself wholly flattened taking the form of a large lobed (i.e. branched and fasciated) ovuliferous scale. In others the ovules carried terminally on the units of such a condensed flattened branching system had become inverted. *Walchia germanica* Florin carried up to five such inverted ovules on distal units of the branching system, Fig. 1 D, while *Pseudovoltzia Liebeana* (Geinitz) Florin carried two (occasionally three) near the base, Fig. 1 E, recalling the structure of the ovuliferous scale of a member of the Pinaceae. In *Ullmannia Bronni* Goeppert the telomic appendages and the axillary dwarf shoot are fused, the whole appearing as a fairly simple flattened scale which bears a single inverted ovule, Fig. 1 F. *Pseudovoltzia* and *Ullmannia* are Upper Permian, the others Lower Permian or Carboniferous.

Except for *Lebachia* accurate published details are not yet available concerning the pollen structure of most of these, although it would appear that the older forms with erect ovules showed the grain type already mentioned for *Lebachia* and shown in Fig. 1 B. With regard to the forms with inverted ovules *Ullmannia*, at least, had a pollen grain, Fig. 1 G, provided with two lateral air-sacs like a modern pine. This interesting fact was given in a personal communication from Dr Florin, obtained through the kind co-operation of the Swedish Consul in Dublin, N. L. Jaenson, to whom a very sincere expression of thanks is due.

The existence in Upper Permian time of an inverted ovule pollinated by a grain with two lateral air-sacs is of great interest. It definitely demonstrates that there took place in the Permian, coupled with the ovular inversion, the change from the primitive grain of *Lebachia* to the modern type, a change simply effected by the suppression of the proximal part of the all-over balloon sac. Many intermediate forms probably existed, such as can be seen in more recent cases. Thus in *Abies nobilis* and *Podocarpus macrophyllus* (Wodehouse 1935), and in specimens of *Pinus silvestris* from certain post-glacial deposits (Florin 1936), there is shown, instead of two lateral sacs, a single bladder completely encircling the distal part of the grain, and intermediates may also occur. The occurrence of three lateral sacs is well known for *Pherosphaera* and *Microcachrys*, and has also been recorded in some species of *Podocarpus*. Many such variants, obvious derivatives from the *Lebachia* type, may have appeared among the Permian conifers with inverted ovules, and

it is here suggested that, when details are available, all the Upper Permian conifers with inverted ovules will prove to have had grains of some such type. It is obvious that these grain types are all merely form variants. They are simply grains provided distally with bladders and all probably function in the same manner. The air-sacs, causing the grains to float in the pollination fluid, facilitate their rise in it *upwards* through the micropylar tube of an *inverted* ovule. At the same time, since the bladders are expansions of the distal part of the grains, these will tend to rise, so oriented that the germinal furrow lies above; thus the part of the grain which makes contact with the nucellus, or at least tends to lie nearest to its surface, is the part from which the pollen tube will later emerge. In the primitive forms with erect ovules, as *Lebachia*, the balloon sac covering a grain tends to keep it floating with the distal pole below, again keeping this area in relation to the nucellus. With ovule inversion the limitation of the air-sac to the distal zone of the grain has, in a very simple way, ensured the reversal of the flotation position of the pollen. The air-sacs seem then to be primarily flotation bladders, a point already emphasised in the account given of pollination in *Pinus* (Doyle & O'Leary 1935 b).

The view has been put forward by Wodehouse (1935) that the function of the lateral air-sacs, in say *Pinus*, is the protection of the germinal furrow on dessication. This view was expressed however before Florin's account of grain structure in the Cordaitales and the Palaeozoic conifers was available. *Lebachia*, for example, has no true germinal furrow, and here the balloon air-sac is solely a flotation device; and in the grains of the two-winged type it is not clear why any such protection should be required in nature. The grains, maturing rapidly in the pollen sac, are spread immediately by wind, and are then either lost or come at once into contact with a moist ovule into whose micropyle they are quickly absorbed. Air-sacs on the pollen sacs, therefore, are taken to be primarily a simple flotation mechanism, although in some modern grains they may function secondarily as protection to the germinal furrow.

Apart from the value of these suggestions it is, at any rate, certain that some conifers of, at least, the Upper Permian had inverted ovules and two-winged grains, and the pollination type, of which *Ullmania* is an example, leads directly to the mechanisms still shown in two of the modern families—the Pinaceae and the Podocarpaceae. Again it is not suggested that these two families necessarily arose from any of the known Permian genera. Ovule inversion and the development of a two-winged grain may not have been achieved by their immediate ancestors till the early Mesozoic. Nor do the extraordinary close similarities that exist between the pollination mechanisms in *Pinus* and *Podocarpus* necessarily mean that these are both to be traced to the same root ancestry. Such questions are not being considered here. But the point is quite definite that the *type* of pollination deemed basal in these two families is not a hypothetical one but one known to have been already developed in the phylum in Permian time and readily derived from a more primitive *Lebachia* type.

Acceptance of the *Lebachia* type of grain as primitive in the conifers, and the derivation from it of the modern two-winged form by reduction, involve naturally the acceptance of Florin's (1936, 1937) criticisms of Wodehouse's (1935) views on primitive grain types not only in the conifers but in the Palaeozoic gymnosperms.

as a whole. It is unnecessary to repeat these criticisms here, but Florin's work shows that many of Wodehouse's statements are erroneous, based as they were on data which at the time were incomplete.

THE PINACEAE

An inverted ovule, an exudation of fluid from the micropyle, and a two-winged grain or some variant of it, functioning as outlined above, are taken to be basal in this family. But within the family several important modifications of the basal type have occurred. There are to be seen, at least, (a) the *Pinus*—*Picea* type, (b) the *Picea orientalis* type, (c) the *Pseudotsuga*—*Larix* type, (d) the *Abies*—*Cedrus* type, (e) the *Tsuga Pattoniana* type, and (f) the ordinary *Tsuga* type. The mechanisms in *Pseudolarix* and *Keteleeria* have not yet been recorded, and are therefore omitted in the discussion which follows. They may prove different from those so far described. Within some of the larger genera minor differences have been described, and it is possible that a comprehensive examination of all the species might bring to light still other mechanisms or important variations on those here listed. But a broad analysis of those already known and described brings out features of much interest.

The types listed are related to four major modifications:—(1) loss of pollination exudate, (2) development of stigmatic devices eventually replacing the primitive method of pollen reception by this exudate, (3) reduction and eventual loss of the air-sacs from the pollen grains, and (4) pollen germination elsewhere than on the nucellus. Further, on various morphological grounds the members of the Pinaceae are often grouped in two series, one including *Pinus*, *Picea*, *Larix*, and *Pseudotsuga*, and the other *Abies*, *Cedrus*, *Tsuga* (*Keteleeria*, and *Pseudolarix*). Each of these two series shows its own special line of modification in pollination mechanism, and in each case the line culminates in a type in which the four major factors are expressed. This can best be seen by a brief review of the salient facts of which the details have been fully recorded in the papers already cited (Doyle and O'Leary, 1935 b, c, Doyle and Kane, 1943). The conclusions are summarized diagrammatically in Fig. 2. Here the two serial lines are based on the Upper Permian *Pseudovoltzia Liebeana*. This is given merely as a convenient and relatively well-known type, the plan of whose cone and ovuliferous scale resembles that in the Pinaceae. It is assumed that its pollination mechanism was that already taken as basal in the family. Its grain, as outlined in section in 2 a, could have been, immaterially, a cleanly two-winged form or one with a circular air-sac round the whole distal zone. In other words, Fig. 2 A is simply meant to represent in a general way what the ovule-bearing apparatus and the pollen grain in the ancestral type of the Pinaceae must have been like.

The *Pinus*—*Pseudotsuga* line, indicated to the left of Fig. 2 A, can first be followed

A. *The Pinus—Picea type.* The pollination mechanism in these two genera seems closest to the basal *Ulmannia*—*Pseudovoltzia* type. The young cones stand rigidly erect so that the ovules are inverted, not only in relation to the cone axis, but in fact (2 B, *Pinus*, and 2 C, *Picea*), while the fluid exudate, suggested by the

dotted line across the micropylar canal in both B and C, and the characteristic two-winged grain $2p$, permit the typical flotation mechanism to be easily seen in operation. Both however have advanced beyond the primitive type by the

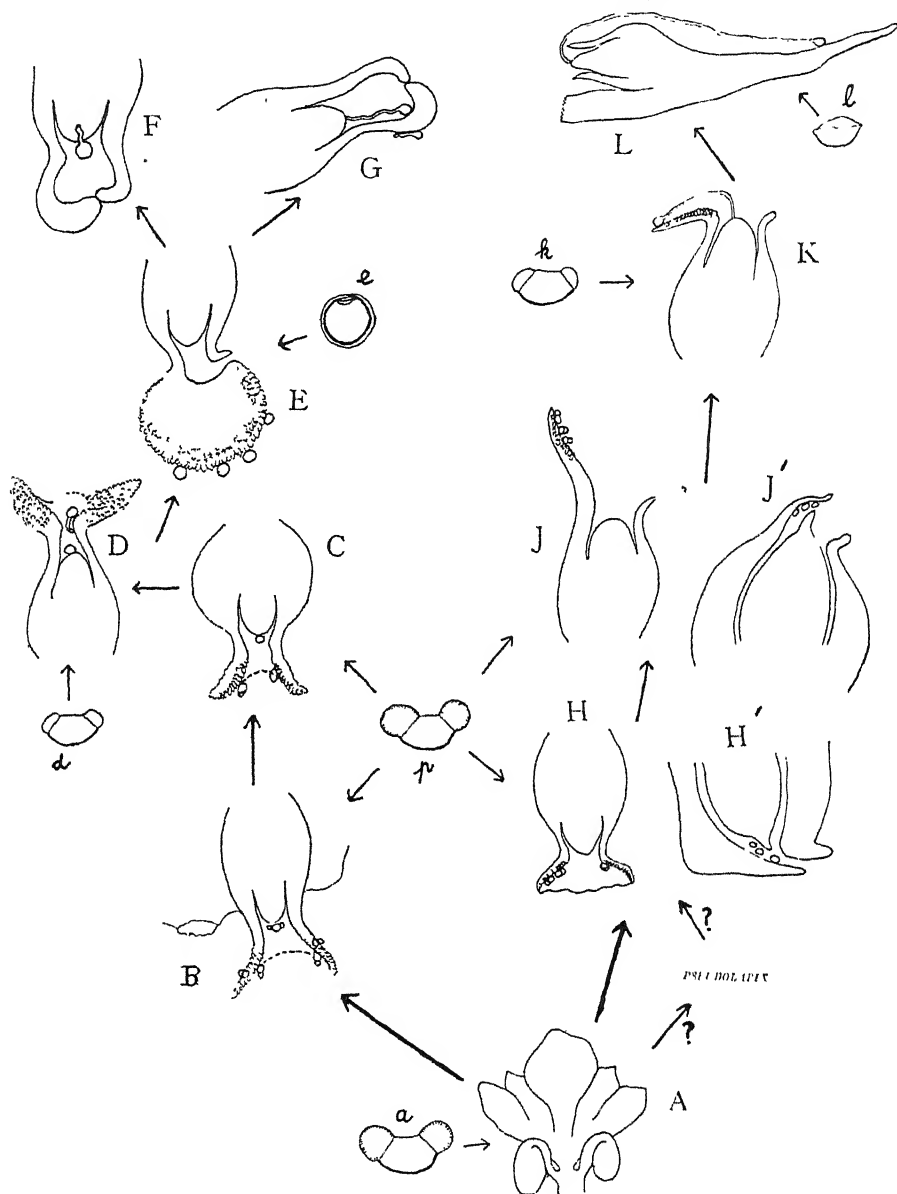


FIG. 2. Pollination behaviour in the two series in the Pinaceae (Diagrammatic). A. *Pseudovoltzia Liebeana* and *a*, its pollen. B. *Pinus* type. C. Common *Picea* type. D. *Picea orientalis* and *d*, its pollen. E. Young *Larix*—*Pseudotsuga* type and *e*, its pollen. F. Later *Larix* type. G. Later *Pseudotsuga* type. H, H'. Young and later stages of *Abies* type. J, J'. Young and later stages of *Cedrus* type. K. *Tsuga Pattoniana* and *k*, its pollen. L. *Eutsuga* type and *l*, its pollen.

In centre, *p*, pollen common to *Pinus*, *Picea*, *Abies* and *Cedrus*. Dotted line across micropyle in B, C, D indicates fluid exudate. For explanation of *Pseudolarix* see text

development from the micropylar edge of stigmatic expansions on which the pollen grains may be caught before the exudation of fluid begins. These are relatively thin in *Pinus*, 2 B, but commonly broader and more succulent in *Picea*, 2 C, which thus shows the tendency to an increasing stigmatic function of the micropyle which is so characteristic of this series.

Both also show an interesting physiological adaptation which may well have been a feature of the Permian types although naturally that can never be determined. The grains are easily wetted by the pollination fluid as it wells up through the micropyle, and so slip readily under its surface. Shortly after such immersion a rapid reabsorption of the fluid takes place. This can be complete in ten minutes or even less.

There are numerous minor variations in this mechanism. The original papers can be consulted for fuller details, but it is worth mentioning that in some cases the pollination exudate may be secreted from the ovules just before the pollen begins to fly. In such cases the pollen may be caught directly in the fluid, as must have occurred in the basal type, and the stigmatic function of the micropyle may thus play a minor role.

B The Picea orientalis type. In all species of *Pinus* and in most of *Picea* the ovulate cones, as already noted, stand rigidly erect, but in *P. orientalis* the young cones hang downwards so that the ovules, though morphologically inverted, lie, in fact, with the micropyles directed upwards, Fig 2 D. Further, the stigmatic function of the micropylar area is much more pronounced, the extensions forming two thick succulent flaps between which only a narrow slit leads into the ovular cavity, from which a normal fluid exudation takes place. But, in relation to the erect position of the ovules, the air-sacs on the grains are much reduced in size, Fig 2 d, so that the grains quickly sink through the slit down to the nucellus. If, however, the grains are numerous some may get massed together and held at the micropylar slit, and some of these may send out a short pollen tube.

The special interest of these features in *P. orientalis*—the departure from the erect position of the cone, the increased stigmatic function, the reduction of the air-sacs, and the pollen germinating, although unsuccessfully, elsewhere than on the nucellus—is that they indicate the manner in which the *Larix*—*Pseudotsuga* type could have arisen.

C. The Larix—Pseudotsuga type. This type shows, then, an extreme advance on the *Picea orientalis* type. The stigmatic function of the micropylar area has here become dominant, a large swollen stigma, Fig. 2 E, developing, which covers the opening. There is no fluid secretion at the time of pollination, and it is clearly of interest in relation to this that the large pollen grains caught externally on the stigma have completely lost their air-sacs, Fig 2 e, and with them the capacity to float. These grains have become so specialised from the primitive type that they show no real vestige of the germinal furrow, the wall being uniform over practically the entire surface. Dubois (1938) however figures a small circular area situated at the distal end and thinner than the rest of the wall. This may represent the last vestige of a furrow.

Inversion and invagination of the stigma brings the grains within the ovular cavity.

Pseudotsuga probably shows a slightly more advanced stage than *Larix*. The cones show no geotropic reaction at pollination but stand at any angle between an erect and horizontal position depending on the directional lie of the actual bud and the branch that bears it. The characteristic drooping position of the older cones is one which is only gradually assumed long after pollination. Further, the pollen remains attached to the inner invaginated surface of the stigma, and there germinates, the long tube growing down the micropylar canal to reach the nucellus, Fig. 2 G. Grains left outside, even those that happened to lodge near by, may send out a short tube. Thus the features associated with the flotation mechanism of the basal type—the erect position of the cones, the fluid secretion, the lateral air-sacs orientating the pollen with the germinal furrow in contact with the nucellus, and the subsequent germination of the grains thereon—have all been obliterated.

Larix, however, still retains the erect position of the cones at pollination, the ovules being thus always strictly inverted, Fig. 2 F. The grains, some time after the inversion of the stigma, become free and lodge on the nucellus, and here only, as in *Pinus* and *Picea*, can they germinate. It is not known how this transfer occurs, since the grains, without air-sacs and heavier than water, must *rise upwards* in the micropylar canal to reach the nucellus. There is, however, a possibility that a delayed fluid secretion within the now closed ovule may, on reabsorption, drag the grains up with it. *Larix* then still retains the basal feature of pollen germination on the nucellus, and possibly also some variant of an ovular secretion, so that *Pseudotsuga*, with the four major factors fully expressed, is, among living forms, the culminating type of this series.

The *Abies*—*Tsuga* series is indicated on the right side of Fig. 2. This series shows phenomena in some respects parallel to those of the *Pinus*—*Pseudotsuga* one, but the end result is different. The whole series in general is characterised by the complete suppression of any fluid exudate at pollination, and by a loss, instead of an intensifying, of the stigmatic function of the micropyle.

D. The *Abies*—*Cedrus* type. The mechanism in *Abies* appears to be nearest to the basal type. Indeed, when it first came under observation it was long thought that a fluid exudation should still take part in the pollination. The cones stand rigidly erect. The ovules, thus inverted in fact, Fig. 2 H, show a definite micropylar canal. The pollen grains, Fig. 2 p, have well developed air-sacs. Thus, although a fluid exudation at pollination is absent, the ovules impress the observer as differing only slightly in their physiological nature from the ancestral secreting type. The micropylar area, though still fairly symmetrical, has become splayed out as a shallow stigmatic funnel on which the grains are caught. The pollen, however, neither lodges on the nucellus nor sends tubes down to it, but the micropyle bends over and the nucellus grows up, Fig. 2 H, to make contact with the nearest grains. In some species, only the grains actually in contact with, or very close to, the upgrowing nucellus germinate, in others, grains a little further out on the stigmatic surface may grow also.

Cedrus shows a somewhat specialized variant of the *Abies* type. The cones do not stand geotropically erect, although they often appear to do so. Actually they stand at right angles to the branch on which they are borne, and on drooping or oblique branches may lie at any angle to the vertical. Once more with the

disappearance of the flotation mechanism the geotropic sensitiveness of the cone is also suppressed. The micropyle area is also very unsymmetrical, expanding as a thin one-sided stigmatic flare, Fig. 2 J, which contracts to a slit on the opposite side. The pollen, Fig. 2 *p*, still carries air-sacs though relatively a little smaller than outlined. The subsequent development however resembles that in *Abies*, the stigmatic flare bending over, and the nucellus pushing up to make contact with the grains, Fig. 2 J¹.

This *Abies* type of behaviour departs so far, in its later phases, from the basal type as to suggest that there must have existed, or possibly still exists, some type intermediate between them. This type might well show a splayed-out stigmatic funnel but still associated with a flotation mechanism. It is just possible that this behaviour may be shown by *Pseudolarix*. Although sufficient cones were never available to permit continued personal observation of the mechanism here at work it is of interest that, although its young ovule closely resembles that of *Abies*, the pollen grains become lodged on the nucellus while it is still deep in the ovule, as if a flotation process were still at work. It is not formally included in Fig. 2.

E The Tsuga Pattoniana or Hesperopeuce type In the genus *Tsuga* a small group of species are included in the special section *Hesperopeuce*, of which the best known is *Tsuga Pattoniana*. In addition to the general character of the section this species differs from those in the other section, *Eutsuga*, in the possession of air-sacs on its pollen. The nature of the grains in other species in *Hesperopeuce* has not been described.

Behaviour here is that of a modified *Cedrus* type. There is, of course, no exudate, and the young cones, showing no geotropic reaction, lie roughly horizontal or droop a little. The micropylar edge expands again as a stigmatic flare, Fig. 2 K, in form intermediate between *Cedrus* and *Abies* but somewhat reflexed, and to it the pollen grains, Fig. 2 *k*, with rather small air-sacs, adhere. There is no bending forwards of the stigmatic flare, which becomes even more strongly reflexed, but at an early stage after pollination the nucellus grows up like a plug into the micropylar canal and protrudes slightly. Further, the possibility of pollen germination at some distance from the nucellus is here a working fact. Grains anywhere on the stigmatic area germinate readily *in situ* the tubes growing directly to penetrate the nucellus. Grains at some distance from the nucellus on the edge of the ovule or on the scale nearby also germinate, and produce quite long tubes which however grow erratically.

Tsuga Pattoniana seems to present a condition which principally differs from the *Cedrus* type only in the simple physiological feature that the pollen can germinate independently of the direct influence of the nucellus. In *Abies* and *Cedrus* a long delay ensues between pollination and growth of the pollen tube, which does not take place until the nucellus has pushed its tapering apex close to the grains. Although not lodged on the nucellus by any flotation mechanism the germination of the grains, in these two genera, is still physiologically related to some nucellar influence just as it must have been in *Lebachia*. In much the same way in the *Pinus*—*Pseudotsuga* series a nucellar influence remains dominant in *Pinus*, *Picea*, and *Larix*, only in the culminating type *Pseudotsuga* has the physiological change been established permitting independent pollen germination,

No satisfactory function can be readily associated with the air-sacs on the pollen in *Abies*, *Cedrus*, and *Tsuga Pattoniana*. They seem to be simply vestigial and harmless structures not yet dropped in development.

F The Eutsuga type This is an advanced development of the last type. It is characterised by the absence of air-sacs from the grains and of any stigmatic functioning of the micropylar area, which is simple and symmetrical, the pointed apex of the nucellus being flush with it. With no stigmatic surface or exudate present the bladderless grains may lodge anywhere on the cone scales, and over these the tubes grow in a surprisingly direct manner, like fungal hyphae, to penetrate the nucellar tip.

Although the grains are bladderless, thus paralleling the stage reached in the other series in *Larix* and *Pseudotsuga*, they are not as specialised as in these two genera. A description and outline drawing of the grain of *Tsuga canadensis* is given by Dubois (1938). The proximal surface is thicker walled and more coarsely ornamented than the distal, and equatorially round the grain runs a slight ridge, Fig. 2 l, which corresponds in position to the more pronounced ridge which encircles the grain in many species of *Abies* just above the upper edge of attachment of the air-sac. It is clear from the account given by Dubois (1938) and from comparison with some fossil *Tsuga* grains referred to by Wodehouse (1938) that the bladderless condition of the pollen in *Eutsuga* is derivative from the two-winged type, they are grains which, geologically speaking, have only recently lost their air-sacs.

It is unlikely, though always possible, that the highly perfected grain in *Larix* or *Pseudotsuga* arose at one step from the typical *Pinus* type. It is of interest then to find, even if in a different line, a grain type, as in *Eutsuga*, intermediate between the two extremes. Some fossil species of *Larix* or *Pseudotsuga*, or possibly even *Picea*, probably had grains of this type.

The behaviour in *Lebachia* is certainly markedly different from that in *Eutsuga*; but this latter type is clearly the result of a series of progressive changes from the former, many steps in the change being still to be seen fixed in other living related types. This *Eutsuga* type is important as it bears directly on conditions in the podocarps and the araucarians. In the latter particularly this behaviour is closely paralleled except that the grain has become almost as specialized as that in *Pseudotsuga*.

Thus from the basal *Ullmannia*—*Pseudovoltzia* type of pollination mechanism two general lines of development appear in the Pinaceae. In both can be seen the loss by the cones of their strong geotropic habit, the loss from the ovule of a fluid exudate, its replacement by a stigmatic functioning of the micropyle, the loss of the air-sacs from the grains, and pollen germination independent of nucellar influence; in other words, the primitive flotation mechanism is ultimately suppressed and replaced in both lines, but in a different manner in each. In one line, the *Pinus*—*Pseudotsuga* line, a stigmatic function of the micropyle, originally developed in association with a still functioning pollination exudate, ultimately became dominant with the loss of this latter; the pollen grain meanwhile becoming so specialized as to lose the primary characters of the ancestral flotation type. In the other line a stigmatic functioning of the micropyle early replaced completely the

suppressed pollination exudate but did not become dominant. Pollen germination, free from nucellar influence, here became the most striking feature, so that ultimately even the stigmatic function of the micropyle was lost, and bladderless grains lodged at random on the cone scales sent long tubes to reach the nucellus.

THE PODOCARPACEAE.

This family shows so much wider a range in vegetative structure and cone form than the Pinaceae that an even greater range of pollination mechanisms might be expected in it. This is probably not the case. Little attention, however, has been given to pollination phenomena in the family, the only published data being those on *Saxegothaea*, summarized and extended by Doyle and O'Leary (1935 a). Actually there is little doubt that the type there shown is exceptional and specialized, but some recent personal observations on *Podocarpus* have proved useful in considering the family as a whole.

Tentative conclusions are illustrated diagrammatically in Fig. 4. As the Podocarpaceae are characterized by having a single, primarily inverted, ovule in relation to a bract the suggested lines in the family are based on the *Ullmannia* type, Fig. 4 A.

A. *The Podocarpus type* Observations have been made on *P. andinus*, one of the simplest living species, and on one of the more advanced forms, *P. nivalis*. As the mechanism in both is essentially the same, and as less complete observations on *P. salignus* and *P. latifolius* seem to agree closely, the pollination type must be reasonably uniform within the genus. The mechanism, while very similar to that of *Pinus*, is a little simpler, and seems indeed to be an actual retention of what that in *Ullmannia* must have been.

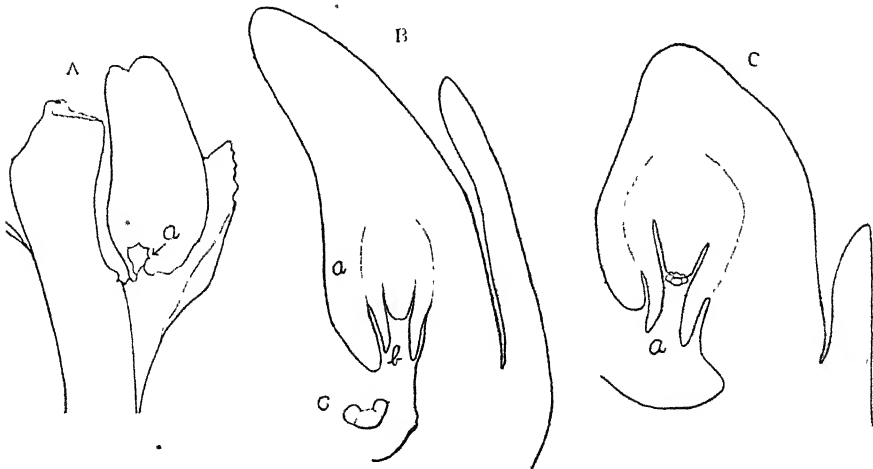


FIG. 3. Strobilus units in *Podocarpus*. A. *P. andinus*, solid view, *a*, arch-like opening under epimatium. B. Same in section, *a* epimatial tissue; *b*, micropyle, *c*, pollen grain. C. *P. nivalis* similar stage in section, *a*, micropyle.

P. andinus, as is well known, has special ovulate branchlets which carry a number of widely spaced bracts. In the axil of each bract lies a stalked axillary structure which bears a single partly embedded and inverted ovule. One such reproductive unit is shown in the solid in Fig. 3 A and in section in Fig. 3 B. The condition is easily derived from the plan of structure of *Ullmannia*, Fig. 4 A. Early growth of the axillary scale, such that the ovule is recessed and covered, can readily produce the so-called epimatium tissue, Fig. 3 Ba, which so completely obscures even the micropyle of the ovule proper in Fig. 3 A. The whole ovulate branchlet is delicately geotropic. In nature it stands erect irrespective of its place of origin on the vegetative stem, the zone of greatest response being just below its lowest ovule. If branchlets are removed and placed in water their lie is, of course, commonly altered but is rectified overnight. When actively growing at pollination time this can be repeated, producing almost an S bend in the lower part, which latter may be only half an inch long. The ovule thus lies with the micropyle pointing almost vertically downwards.

P. nivalis shows a typical *Eupodocarpus* branchlet carrying two, or only one, ovule. The branchlets are quite short in this species, but show an equally delicate geotropic response, which occurs most markedly at the junction of the short narrow stalk and the swollen podocarpean foot. Clearly here too the inverted ovules point vertically downwards.

The micropyle in both is symmetrical and simple showing no stigmatic tendency. This micropyle area is, of course, double. The embedded ovule is completely covered by the epimatium, the lower edge of which forms a simple arch-like channel, Fig. 3 Aa, within which lies, in *P. andinus*, the tubular micropyle proper, Fig. 3 Bb. In *P. nivalis*, Fig. 3 C, the micropyle *a* projects beyond the arch as a short reddish tube, but it is not known if such minor differences affect the behaviour in any way. A copious pollination exudate is secreted in both, and in this the pollen grains are caught. These have two well developed lateral bladders, Fig. 3 Bc, and float upwards orientated as in *Pinus*. Shortly after immersion of the grains, the fluid, again as in *Pinus*, is rapidly reabsorbed. The secretion seems to be continuous, not rhythmical, as in some species of *Pinus*, so that if pollen is not received at a suitable stage the drop increases and may even, under laboratory conditions, run down the side of the branchlet. Retraction under these conditions is slower or erratic, but, owing to the evaporating power of the air in early June when pollination takes place, this effect is seldom seen in nature in the open.

Clearly then the mechanism in *Podocarpus* is of the basal flotation type, lacking even any early stigmatic tendency. There is every reason to accept the view, based on gynostrobilus structure and features of gametophytic and embryological development, that *P. andinus* is the simplest living podocarp. It would appear then that the simple flotation mechanism is basal in the Podocarpaceae also, and derived, with little change, from ancestral Permian forms of the *Ullmannia* type.

B. The Saxegothaea type. Here the behaviour is quite different but is comparable to that in the *Eutsuga* type. There is no fluid secretion and no special geotropic response in the cones. The pollen grains, almost spherical and without bladders, Fig. 4 g, fall on the cone bracts, and, if within a certain range of the ovule, send out tubes, Fig. 4 G, to reach the nucellus, which projects as a marked

bulge well beyond the micropyle. It was at one time thought (Norén, 1908) that this was a stigmatic projection for pollen reception, but it is now clear (Doyle and O'Leary 1935 a) that it is developed for the reception of the tips of the pollen tubes, which, having penetrated it, remain dormant for about nine months.

Although no intermediate between *Podocarpus* and *Saxegothaea* are to be found among living forms there can be little doubt, in view of the conditions in *Podocarpus* and *Pinus*, that this behaviour in *Saxegothaea* represents in the family of the Podocarpaceae a development similar to that shown by the *Eutsuga* species in the Pinaceae. There are, of course, obvious differences, which need no repetition here, but they are only differences in detail. The lack of any geotropic response in the cones, the loss of the pollination exudate, the loss of the bladders from the grains, the growth of the tubes on the cone scales, these are all repeated. It is of further interest in this connection that, although *Saxegothaea* has retained many primitive features, its cone organization is best interpreted as an advanced derivative from the simple *Podocarpus* type marked by fusion and reduction of parts. Specialization in pollination behaviour has thus accompanied specialization in the cone.

When fewer data were available many earlier workers (*ie* Thomson 1909, Stiles 1912), impressed by the apparently simple nature of the grain in *Saxegothaea*, were of the opinion that this bladderless type of pollen was primitive in the podocarps, the winged grains of other genera being thus a new development in the family. Sinnott (1913), however, among others, held, on general comparative grounds, that the air-sacs were ancestral features now lost in *Saxegothaea*, a view recently supported also by Wodehouse (1935). The comparisons here drawn between the two series of pollination types, *Pseudovoltzia*—*Pinus*—*Eutsuga* and *Ullmannia*—*Podocarpus*—*Saxegothaea*, definitely establish the air-sacs as ancestral and basal in the podocarps.

C. Other podocarpean genera. Unfortunately no details are available concerning pollination behaviour in any of the genera other than *Podocarpus* and *Saxegothaea*, but from what is known of the structure of ovules and pollen grains it appears probable that behaviour in this family is more constant than in the Pinaceae, and that some variant of the podocarpoid flotation mechanism is characteristic of all the genera except *Saxegothaea* and possibly *Phyllocladus*.

Thus, in many species of *Dacrydium* the ovules, when young, seem to be definitely inverted in relation to the vertical, although the actual lie of the ovule may change rapidly with age. Since, further, most species of *Dacrydium* have normal two-winged grains which lodge on the nucellus, and since the micropyle seems to be, commonly, symmetrical and non-stigmatic, it is probable that we are here dealing with a simple flotation mechanism as in *Podocarpus*. In Fig. 4, therefore, B and the grain *b* represent in general both *Podocarpus* and *Dacrydium* in relation to the ancestral type suggested by *Ullmannia*. It is more than probable, however, that when details are available this single grouping will be shown to include a number of derivative mechanisms. This is suggested in Fig. 4 by the arrows and queries leading from the top right of B.

The ovule of *Microcachrys*, Fig. 4 D, as shown in Lawson's (1923 a) figures, is at least obliquely inverted with a long extended micropylar tube directed vertically downwards. The pollen, 4 *d*, which lodges on the nucellus, has typically three

large bladders round the distal part of the grain. For these reasons, and also because *Microcachrys* almost certainly is a member of a line in the podocarps distinct from the *Podocarpus*—*Dacrydium* one, it is shown separately in Fig. 4, although in general the pollination behaviour must conform to the basal podocarpoid type

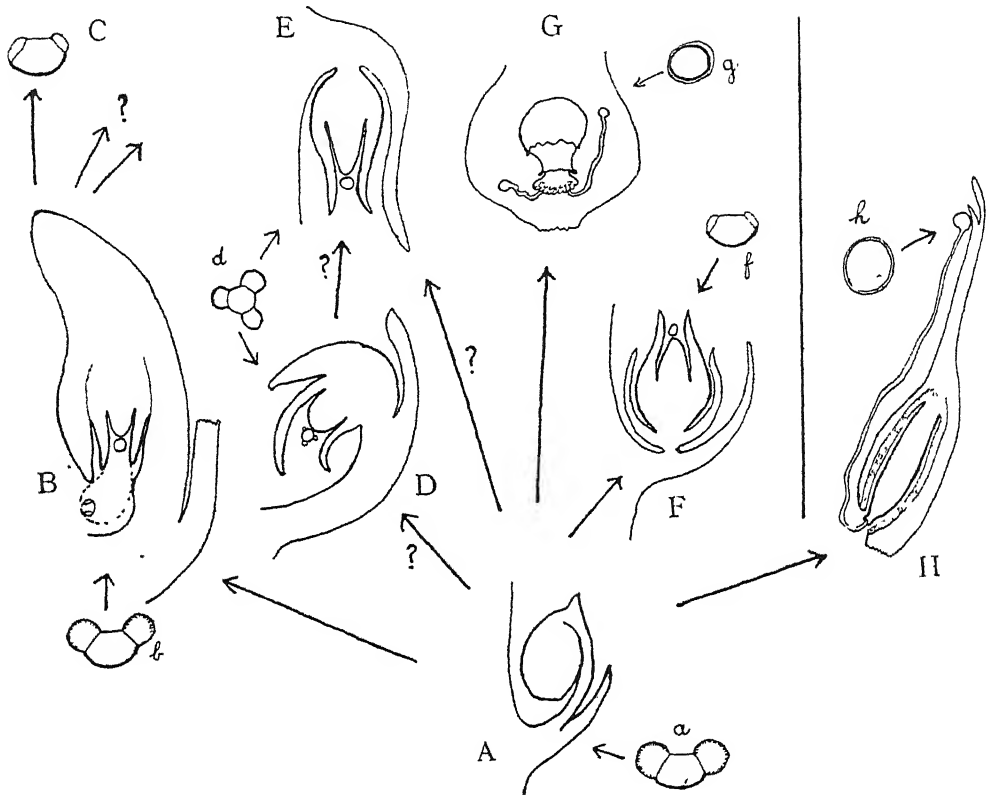


FIG. 4. Pollination behaviour in Podocarpaceae, B—G. and Araucariaceae, H. (Diagrammatic). A. *Ullmannia Brownii* and *a* its pollen. B. The *Podocarpus*—*Dacrydium* type and *b* its pollen; dotted line indicates pollination exudate. C. *Dacrydium* pollen type with rudimentary wings. D. *Microcachrys*. E. *Pherosphaera* *d* pollen of D and E. F. *Phyllocladus* and *f*, its pollen. G. *Saxegothaea*, and *g*, its pollen. H. *Araucaria* type and *h*, its pollen.

Arrows and query to right of C indicate possible but undescribed *Dacrydium* derivatives.

Acmophyle is omitted, as its behaviour is quite uncertain.

Pherosphaera, as a genus, may be a survivor of a distinct line or a derivative of the *Microcachrys* line as shown by the overlapping of their natural areas of distribution, the similarity in habit, the common possession of a three-bladdered grain, 4 *d*, and other features. This uncertainty is suggested by the query mark on the lines connecting A with D and E in the diagram, Fig. 4. But its pollination mechanism seems to be again essentially the simple podocarpoid flotation type. In *Pherosphaera* the axillary ovuliferous structure of the *Ullmannia*—*Podocarpus* plan has been reduced to a single morphologically erect ovule. But, according to Pilger (1926, Fig. 125), the young cone is inverted so that the actual ovules and

micropyles are again directed downwards, Fig. 4 E. The three-winged grains lodge on the micropyle (Lawson, 1923 b). Apparently the reduction in the cone structure has been accompanied by an inversion of the whole cone thus permitting the retention of the ancestral flotation mechanism.

The behaviour in *Acmopyle* is more problematic and is therefore omitted from Fig. 4. The grain has two well-developed air-sacs, but the form and lie of the young ovule of this rare plant are unknown. The name is derived from the form of the maturing ovule, in which the micropyle points upwards. At pollination however, the young ovule may well be strictly inverted, assuming the maturer position by later uneven growth, as so commonly occurs in *Dacrydium*. If so, a typical podocarpoid flotation mechanism would probably be operative in this case. The only figure available, however, of pollen in relation to the nucellus, a simple line drawing (Sahni 1920), shows an upgrowth of the apex of the nucellus into the micropyle with the grain proper separated from its surface by a length of the pollen tube; a condition which might indicate a behaviour along the lines shown by *Abies* in the Pinaceae.

Apart from the at present uncertain relationships of the genera mentioned they all seem to have retained the ancestral type of pollination mechanism or some simple variation of it with the doubtful exception of *Acmopyle*. Other forms, however, as well as *Saxegothaea*, seem to have departed more from the type. Thus in *Dacrydium*, as in *Tsuga*, an intrageneric modification in the pollen grains has taken place, as two species, at least, are reported as showing only rudimentary air-sacs—*D. Gibbsiae* (Wodehouse 1935) and *D. cupressinum* (Sinnott 1913). In what way the pollination mechanism is affected, if at all, is unknown. The fact is indicated in Fig. 4 C and the nearby query marks. Further, in *Phyllocladus* the ovule seems to stand at least obliquely vertical and thus with the micropyle above, Fig. 4 F. The pollen grains, with only rudimentary wings, Fig. 4 f, undoubtedly lodge on the nucellus, and, as in *Picea orientalis*, may do so by sinking in the pollination fluid, which is subsequently reabsorbed; but again the actual behaviour is unknown. However, even these modifications in *Dacrydium* and *Phyllocladus*, in view of the known cases in the Pinaceae, must also be considered direct derivatives from the ancestral flotation type.

The marked difference in the gynostrobilus in living podocarps, coupled with many features in gametophytic and embryological development, suggest, as indicated in Fig. 4, an early divergence of several lines from the primitive podocarpoid stock. Along several of these lines the basal Palaeozoic type of pollination mechanism seems to have been retained. It can be seen, at least in principle, in the lines represented to-day by *Podocarpus*, the related *Dacrydium*, *Microcachrys*, *Pherosphaera*, and possibly in *Acmopyle*. Within *Dacrydium* modifications of an unknown type have probably occurred. As the three-bladdered condition in *Microcachrys* and *Pherosphaera* does not directly affect the pollination mechanism, the grain floating and orientating itself as in the normal, it need not here be further discussed, although it may obviously bear on general questions of intergeneric relationships. It does suggest however that the primitive pollen grain in the podocarps may have possessed a bladder all round the distal half, a bladder which evolved into two air-sacs

in some lines and into three (or more) in others. As in the Pinaceae the grain figured in Fig. 4 *a* may be taken as one with two lateral air-sacs or one with a continuous circular bladder seen in section.

The only major modifications known to have become established in the family are those shown in *Phyllocladus* and *Saxegothaea*. The exact nature of the former is obscure, but it is clear from the preceding discussion that the behaviour in *Saxegothaea* is exceptional. It is an advanced specialized type but quite comparable to that developed in the Pinaceae in *Eutsuga*.

Only in *Podocarpus* and *Saxegothaea* have the actual facts been observed and recorded. Proper conclusions for the family as a whole can only be come to when the data for the other genera and divergent types have similarly been put on record. And this can only be done by observations, *in situ* in the field, of the actual processes during the pollination period, which is usually of short duration, often not more than a week.

THE ARAUCARIACEAE

The pollination mechanism in the araucarians, *Araucaria* and *Agathis*, first described by Thomson (1907), is now well known. It is essentially similar to the *Eutsuga* type. The pollen grains, Fig. 4 *b*, which are without air-sacs, lodge on the cone scales, and the tubes grow towards the ovule, the apex of the nucellus projecting to receive them, Fig. 4 *H*. The grains, however, are more advanced than in the hemlocks, and tube growth is more vigorous.

When first described this phenomenon roused much interest, and many earlier workers, impressed by the archaic characters supposed to be shown by the family, discussed the possibility of this type of pollination being basal in the conifers as a whole. This idea has been, perhaps, best elaborated by Burlingame (1915). If, he argued, the gymnospermous ancestors of the conifers all had a pollen chamber for reception of grains caught by a pollination drop exuded from the micropyle, as is still shown by living Cycads and *Ginkgo*, it is difficult to see how pollen reception on the cone scales and its germination there could have arisen. He suggested that such would have been a highly disadvantageous change. For the Pteridospermae, with exposed ovules on open fronds, direct ovule reception of the pollen would appear to have been the only possible type, but for the ancestral conifers he postulated that, in these, cone formation, the protection of ovules by spreading scales, was developing at the same time as the seed was being evolved. In spite of the fact that the ovules of all the then known fossil gymnosperms possessed a pollen chamber mechanism, he further postulated the existence of other, presumably earlier, types, in which this feature was lacking. In these, he suggested that pollen reception could well have been on the scales of the cones then in process of organization. In subsequent modification only grains closer, and then still closer, to the ovule could achieve successful tube growth, and so direct ovular reception was finally effected. Such ovular reception became characteristic of all the early conifer families except the araucarians, which, he claimed, still retained the archaic type of cone reception—the so-called protosiphonogamous mechanism.

In the light of present knowledge it is hardly necessary to criticise this view except in very general terms. Undoubtedly, we do not know how fertilization

was effected in the pterygynnosperm phyla when the gynosporangium was becoming the ovule, but that at the moment is immaterial, as it refers to an earlier problem which does not here arise. It seems quite definite, however, when Florin's work is added to previous data, that the immediate conifer ancestors, in Carboniferous and Permian time, all had direct ovular reception of the pollen, so that the araucarian type must have derived from this. Knowing, as we do, so little of the physiology and biology of these pollination processes how can any one modification be dismissed as a highly disadvantageous change? It cannot be denied, for example, that scale reception and germination in *Eutsuga* have been derived from a type with direct ovular reception, and the hemlocks are a successful and widespread group. The real problem would appear to be concerned, not with modifications in the physiological behaviour of pollen tubes, but rather with the origin of any pollen tube at all. The fossil record is still too obscure for discussion of that problem, but it is tempting to suggest that the development of the two-winged condition in the ancestral grain, orientating a relatively thin germinal area into contact with nucellar tissue, may have been related to the change in that area from a fission zone to a zone which underwent extension growth as in a root hair.

The araucarians, like the podocarps, show a single inverted ovule, and the pollination mechanism of the ancestral type must have closely resembled that of *Ullmannia*. In Fig 4, therefore, the araucarian type, H, is shown as related to the same basal *Ullmannia* type, A, as the Podocarpaceae. Indeed *Ullmannia* itself, in habit and in cone, points directly to the araucarian form, which can readily be derived from it by reduction of the axillary ovuliferous scale and its fusion with the subtending bract. Further, Wodehouse (1935) describes, on the grain of some species of *Araucaria*, traces of a vestigial germinal furrow indicative of a grain in which air-sacs have but recently been suppressed. The grains of the more advanced genus, *Agathis*, do not show even this vestigial trace; they are on the same level of advance as *Pseudotsuga*. The bladderless condition here is thus comparable with that obviously developed by a similar loss of bladders in *Larix*, *Pseudotsuga*, *Tsuga*, and *Saxegothaea*.

Although, then, the araucarians may have retained many features of an archaic type it is suggested that they show a pollination behaviour of an extremely advanced character. The mechanism, derived ancestrally from the same basal flotation type as that in the Pinaceae and the Podocarpaceae, has developed in a manner comparable to that shown by *Eutsuga*. Advance has gone even farther in the araucarians as the grain is more specialized and the pollen tubes show greater power of growth. The changes in the Pinaceae leading to the *Eutsuga* type may have been quite recent, but in the Araucariaceae the evolution must have taken place much earlier, the intermediate species or genera being now extinct. Such intermediate araucarian types, comparable perhaps to *Cedrus*, *Abies*, or *Tsuga Pattoniana*, are probably waiting discovery in the early Mesozoic strata.

THE OTHER FAMILIES.

The Taxodiaceae, Cupressaceae, and Taxaceae (including *Cephalotaxus*), all show the simple pollination-drop mechanism with wingless grains, a type shared

also by the other living gymnosperms. In these the micropyle is symmetrical and non-stigmatic, and the pollination fluid exudes through it as a round glistening drop in which the grains are caught, the grains lacking not only air-sacs but, in most cases, even any vestige of a germinal furrow or pore.

Relatively little critical examination has been made of this mechanism. Tison (1911) gives some interesting data, but his observations are still rather general. More precise examination over a wider field is needed to elucidate the nature of the variations which undoubtedly occur. Apparently all these wingless grains sink in water or in fluid of the concentration of the drop. It might be expected then that the ovules would stand universally erect and so permit the grains to roll down the micropylar canal to the nucellus. But in many—*Taxus*, *Cryptomeria*, species of *Cupressus*, and others—although the young ovules are morphologically erect the cones are strongly inverted, so that, in fact, the micropyle and drop are turned downwards. The grains when received first fall away from the micropyle opening. In others the young ovules may be, in fact, horizontal, oblique, or erect. How the grains reach the nucellus, especially of the inverted ovules, is not clear, although there is some evidence, also noted by Tison (1911), that rapid retraction of the fluid may occur after a certain period. Again, in most, probably in all, of the grains concerned the intine is thick and can swell enormously in water throwing off the extine. In which species this occurs *in* the drop before retraction and in which only when the grains have reached the nucellus has still to be determined. The swelling may be rapid or slow, but some grains which swell slowly in water will, after a brief immersion in the pollination fluid followed by transfer to water, show an almost instantaneous swelling. Such points are merely touched on here to indicate the nature of the problems which arise and which need solution in relation to the detailed working of the variants in the mechanism. But in all cases the pollen grain becomes lodged on the nucellus, and there germinates.

Apart from the details of such problems, whose solution merely depends on suitable simple experiments and close observation of living material at the pollination period, a relation of the mechanism in general to the Permo-Carboniferous types may be suggested. The ovules themselves have changed little from the *Lebachia*—*Ernestiodendron* type, except of course in the feature common to all living conifers, the absence of a pollen chamber. The micropyle is fairly symmetrical, and lacks, as far as is known or recorded, any stigmatic device, but readily permits the retention and display of the characteristic drop. The principal change from the ancestral type, then, is in the grain characterized by the complete suppression of any flotation bladder, the absence of any vestige of a germinal furrow, and the thick swelling intine. Now there is no evidence that this grain ever passed through a clean-cut two-winged stage or one of its variants. That grain type seems primarily related to an inverted ovule associated with a cone axis which is sensitively geotropic and which maintains the inverted ovule more or less accurately in a vertical position. The families showing the simple pollination-drop type do not show typically a strictly inverted ovule at pollination. They may thus be related to some Permo-Carboniferous types in which the ovules remained erect. The grain then would be simply that of *Lebachia* with the balloon sac completely suppressed. The grain of *Lebachia* can hardly be said to possess a true germinal

"furrow," the distal germinal pole, not covered by the sac, being a small and more or less circular area. Any vestige of this area has completely disappeared in most of the grains of these living types, but it may well be that the small circular papillate area shown in the pollen of *Sequoia* and *Cryptomeria* represents the distal pole of the *Lebachia* grain.

These suggestions can, at present, be merely tentative. The taxads and the taxodinean conifers belong to two very divergent lines whose Permian ancestors may have been different. The evolution of pollination mechanisms, and ovular position in relation thereto, may have then followed quite a different path in the two groups. Thus, in *Phyllocladus* in the Podocarpaceae, complete suppression of the two rudimentary air-sacs could give a wingless grain associated with a symmetrical micropyle and a typical pollination drop, although, ancestrally, *Phyllocladus* must have been derived from a form with inverted ovules, two-winged grains, and a flotation mechanism like *Podocarpus*. In some of the living groups, now showing the simple pollination-drop mechanism, development may have taken place along some such lines. But, as living intermediates, such as illustrate progress in the Pinaceae and the Podocarpaceae, have not been observed in the families here concerned, and as their early fossil history has still to be determined with reasonable certainty, decision on the points here raised must await the uncovering of further fossil evidence. The possibilities have therefore not been illustrated by any diagram.

SUMMARY.

This paper deals with the relations of pollination mechanisms only, not with the relations of individual genera. Basal to the conifers as a whole is the type shown by Upper Carboniferous and Lower Permian forms such as species of *Lebachia* and *Ernestiodendron*. An erect ovule receives, in a fluid exudate from a simple micropyle, a pollen grain entirely covered by a large air-sac except for a small distal area. In the Upper Permian, as shown by species of *Ullmannia* and *Walchia*, there developed inverted ovules associated with grains of a modern type, i.e. with two distal air-sacs derived by suppression of the proximal part of the balloon sac. This type is basal in the Pinaceae, Podocarpaceae, and Araucariaceae. The air-sacs are flotation devices which float the grains up through the pollination fluid in the micropylar canal of an inverted ovule, and tend to orient them with the germinal furrow in contact with the nucellus.

From this two lines appear in the Pinaceae both ultimately showing loss of exudate, stigmatic development of the micropylar area, suppression of air-sacs from the grains, and pollen germination elsewhere than on the nucellus. The end result differs in the two lines. In the first—*Pinus*, *Picea*, *Larix*, *Pseudotsuga*—the ovular exudate is ultimately replaced by an increasing dominance of the stigmatic tendency culminating in *Pseudotsuga*, where however germination of the now wingless grains is still intraovular but not on the nucellus. In the second—*Pseudolarix* (?), *Abies*, *Cedrus*, *Tsuga*—the exudate is early suppressed, and the stigmatic function of the micropyle, never very marked, is ultimately replaced in *Tsuga* by pollen reception on the cone scales, over which long tubes grow from the wingless grains. *Tsuga Pattoniana*, with winged grains, is an interesting intermediate type.

In the Podocarpaceae *Podocarpus* shows a typical Upper Permian type with simple micropyle and no stigmatic tendency. In *Saxcegothaea* scale reception and the germination thereon of wingless pollen has been achieved as in *Tsuga*. Most of the other divergent lines in the podocarps seem to have retained the basal *Podocarpus* type, or some variant of it, except *Phyllocladus* and some species of *Dacrydium*, which have only rudimentary air-sacs on the grains. Details of the pollination behaviour in these have not been observed.

The Araucariaceae, though derived from forms showing the Upper Permian flotation mechanism, show scale reception and germination thereon of wingless grains, *i.e.* development has paralleled that in *Tsuga* in the Pinaceae but is somewhat more advanced. Intermediate types are extinct.

The other families, Taxaceae (including *Cephalotaxus*), Cupressaceae and Taxodiaceae, show the simple pollination-drop type with wingless grains. Few details are available as to the actual functioning in this type or concerning the variants of it which may occur. The early fossil lines for these families are not clear. But tentative relations with the *Lebachia* pollination type are suggested.

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BIOCHEMICAL ANALYSES WITH THE SPEKKER ABSORPTIOMETER

I. ESTIMATION OF UREA, CITRULLINE, ALLANTOIN AND RELATED
CARBAMIDO COMPOUNDS

By

EINHART KAWERAU, M.B., M.Sc.

Department of Biochemistry, Trinity College, Dublin

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THE carbamido-diacetyl reaction described by Fearon (1939) has been used for the direct colorimetric estimation of citrulline by Gornall and Hunter (1941) and Archibald (1944), and for the estimation of urea by Abelin (1940), Ormsby (1942), Barker (1944), and Archibald (1945). No fundamental alteration was made by these workers, although each one adopted a different procedure. During an extended study of the test in this laboratory, the optimum conditions for the reaction were determined with the Spekker photoelectric absorptiometer (Adam Hilger & Co., Ltd.), and calibration curves for urea, methyl urea, citrulline, and allantoin were constructed.

The test essentially consists in boiling the substrate with strong acid and diacetyl, and intensifying the resulting pigment by a suitable oxidiser. With urea, a yellow pigment develops, with a mono-substituted urea, the pigment is red. The urea pigment in low concentration has a green tinge, the red substituted urea pigment in high concentration has a yellow component that causes the pigment to appear almost orange. Maximum absorption for the urea colour is at $475\text{ m}\mu$, for the red pigment it is between $485\text{--}495\text{ m}\mu$ (Barker, 1944). In order to obtain maximum colour intensity, the pigment must be oxidised, but if over oxidised the pigment rapidly fades. With certain precautions satisfactory results are obtained by addition of the minimum necessary amount of 1 per cent potassium persulphate, a method which was originally used by Fearon, and has been adopted by subsequent workers.

For the purpose of quantitative estimation the test has two objections. Firstly, and most important, persulphate oxidation is difficult to control. Using only traces of persulphate, bleaching rapidly overtakes maximum colour formation; the red pigment especially is sensitive to the hydrogen peroxide liberated from the persulphate. Abelin (1940) overcomes the difficulty by using no oxidiser at all. Fearon found arsenic acid a suitable oxidiser (personal communication). The acid acts only in the boiling mixture and ceases to oxidise vigorously when the solution is cooled to room temperature. Phosphoric acid acts in a similar way. It was introduced into the test by Archibald (1945). This simplifies the procedure and lessens the oxidation hazard.

A second objection is raised by Ormsby (1942), who states that every time a new batch of diacetyl is used a new curve has to be constructed. Using diacetyl monoxime as a source of diacetyl it is found that in 3 per cent. concentration the

reagent tends to precipitate and form a brown polymer, thereby altering in strength. These difficulties have been overcome, and a diacetyl reagent is obtainable that gives identical curves with different batches of diacetyl monoxime. Archibald (1945) uses α -isonitrosopropiophenone instead of diacetyl monoxime, and claims that it is a more specific reagent for urea, however, diacetyl monoxime is a much more sensitive reagent than α -isonitrosopropiophenone and is preferred by the present author. Satisfactory results can be obtained with either reagent.

OUTLINE OF METHOD

Apparatus. A Spekker photoelectric absorptiometer, or any other type of absorptiometer may be used. The fused quartz cups supplied with the Spekker instrument are unsuitable for immersion in a water-bath. The pigment is generated in 9 ml long-stem Folin-Wu tubes (as used for blood-sugar estimations). The long stems conveniently act as condensers. A metal rack can be constructed to hold six tubes upright in a one litre Pyrex beaker. The edge of the lower plate of the rack is turned down so as to raise it a sufficient distance to prevent the tubes making contact with the floor of the beaker. As an alternative, an electric immersion heater in a litre beaker can be used, but care must be taken that the tubes make no contact with the metal of the heater.

Colour Filters. The spectrum blue gelatine filter (No. 602) with a maximum transmission close to $475\text{ m}\mu$ is used for the yellow pigment (urea, allantoin). The blue-green gelatine filter (No. 603) with a maximum transmission at $500\text{ m}\mu$ is used for the red pigment (citrulline, methyl urea). If no gelatine filters are available glass filter No. 7 should be used for both types of pigment.

Reagents. The following oxidising acid mixtures give satisfactory results:

(A) One volume of concentrated sulphuric acid is added to three volumes of syrupy phosphoric acid (Sp.gr. 1.75). Any turbidity in the mixture settles out on standing, and the clear supernatant liquid can be poured off for use.

(B) 50 ml. of concentrated sulphuric acid are added to 50 ml. of 20 per cent. arsenic acid.

(C) Ten grams of arsenic acid, or the molecular equivalent of sodium arsenate are dissolved in 100 ml. of concentrated (36 per cent) hydrochloric acid.

The following diacetyl reagent keeps satisfactorily for at least two months at room temperature and shows no discolouration or precipitate. New batches of diacetyl reagent have given identical results. Dissolve one gram of diacetyl monoxime in 100 ml. of 5 per cent acetic acid. If the diacetyl monoxime is not perfectly white, the solution can be decolourised with norite charcoal.

Standard Solutions. Only pure analytical reagents were used. The citrulline was prepared according to the method of Fox (1938). The substances are dried before weighing, volumetric solutions containing 30 mg. are made up with glass-distilled water; a few drops of toluene are added as preservative. From these stock solutions dilutions of 1:10 and of 1:2 were prepared. The 1:10 dilution contains $15\text{ }\mu\text{g}$ in 0.5 ml., the 1:2 dilution contains $75\text{ }\mu\text{g}$ in 0.5 ml.

General Considerations. Both types of pigment do not obey the Beer-Lambert absorption laws, therefore a graph where concentration is plotted against density shows a curve. The only part of the curve for which one could substitute a straight line without involving an error greater than 1 per cent. is that from $30\text{ }\mu\text{g}$ to $90\text{ }\mu\text{g}$.

in the case of urea and methyl urea, and from 75 μg to 180 μg for citrulline. Values below or above these ranges lie on definite curves, bent in opposite directions. It is essential therefore to construct an accurate curve. At least 8 points should be determined, from 0 μg to 90 μg they should be taken at 15 μg intervals, and from 90 μg to 150 μg at 30 μg intervals.

The procedure now is as follows. Measure into the Folin-Wu tube the amount of standard solution necessary to yield one of the pre-determined points on the curve, now add 2 ml of the diacetyl reagent and either 3 ml of acid mixture (A) or 4 ml of acid mixtures (B) or (C). The volume is made up to the 9 ml mark with distilled water. The tube is stoppered and inverted to mix the contents thoroughly, and then put into a vigorously boiling waterbath.

The boiling is maintained for 20 minutes, if urea or allantoin is estimated, but only for 15 minutes in the case of citrulline and methyl urea. If the level of the water bath reaches just to the 9 ml mark the upper part of the tubes remain sufficiently cool to act as efficient condensers, and there is no need to adjust the volume after boiling.

After removal from the waterbath, the tubes are put into running cold water for approximately 2 minutes, wiped dry, and poured into the comparator cups. If these cups are wet they can be rinsed with a small amount of the fluid from the Folin-Wu tubes, since the former only hold 8 ml. The colour intensity is measured at once with the Spekker absorptiometer, using the appropriate filters. No fading of the colour occurs for at least 30 minutes. The standard values obtained for urea, methyl urea, citrulline, and allantoin are given in Table 1.

Variable Test Conditions. The method just described was adopted after every reactant in the test was carefully checked. Any variation in the composition of the reagents, or in the test method leads to different results. The method for optimum colour production therefore could only be determined after varying each individual component of the test. Some of the results are grouped together in Table 2.

The importance of using the correct amount of diacetyl is outstanding. Slight errors in the amounts of diacetyl give rise to large errors in colour values. Using excess of diacetyl avoids this hazard. Acid concentration and boiling time are two other important factors. The optimum acid concentration was found to be 4 ml. for the hydrochloric acid mixture, and somewhat less than 4 ml. for the other acids. An increase beyond 4 ml. results in somewhat reduced colour values, especially when the substrate is in high concentration. Table 2 shows that optimum colour production has not been reached after boiling for 20 minutes, but the proportional increase in colour value after this time was not thought sufficient to lengthen the period of boiling as a routine measure.

Archibald (1944) recommends that the procedure should be carried out in the dark, since both types of pigment after development are photosensitive. On exposure to light the colour intensity gradually increases. In the present author's experience this does not occur at all when acid mixture (C) is used (see Table 2a) and is only very slight with acid mixture (A) after allowing the solutions to stand for 48 hours; but for the practical conduct of the test this is of no importance. Archibald's observation is probably due to the failure to use excess of diacetyl in the test.

TABLE I.
Density Values

| μg | Urea Acid (A) | Urea Acid (C) | Allantoin Acid (A) | Methyl Urea Acid (C) | Citrulline Acid (C) |
|---------------|------------------|------------------|-----------------------|-------------------------|------------------------|
| 15 | 0.07 | 0.07 | <0.01 | 0.04 | 0.01 |
| 30 | 0.22 | 0.20 | <0.01 | 0.13 | 0.05 |
| 45 | — | 0.36 | <0.01 | 0.28 | 0.098 |
| 60 | 0.62 | 0.51 | 0.01 | 0.42 | 0.14 |
| 75 | 0.84 | 0.67 | <0.02 | 0.57 | 0.21 |
| 90 | 1.02 | 0.80 | 0.02 | 0.70 | 0.28 |
| 105 | 1.24 | 0.94 | — | 0.82 | 0.35 |
| 120 | 1.31 | 1.05 | — | 0.92 | 0.42 |
| 135 | — | 1.15 | — | 1.01 | 0.49 |
| 150 | 1.55 | 1.26 | — | 1.08 | 0.55 |
| 165 | — | 1.34 | — | 1.15 | 0.62 |
| 180 | 1.69 | 1.40 | — | 1.20 | 0.68 |
| 210 | 1.75 | 1.50 | — | 1.30 | 0.80 |
| 240 | 1.78 | 1.56 | — | 1.38 | 0.90 |
| 270 | — | 1.60 | — | 1.44 | 0.98 |

TABLE 2*.

I. Varying the amount of diacetyl for 30 μg Urea Acid Mixture (A).
ml. of 1% diacetyl . 0.2 0.6 0.8 1.0 1.2 1.4 1.8 2.0 2.4

| | | | | | | | | | |
|---------------|------|------|------|------|------|------|------|------|------|
| Density Value | 0.08 | 0.30 | 0.36 | 0.42 | 0.46 | 0.48 | 0.50 | 0.51 | 0.52 |
|---------------|------|------|------|------|------|------|------|------|------|

II. Varying the amount of HCl for 55 μg Urea. Same amount of oxidiser.
ml. conc. HCl 1 2 3 4 5

| | | | | | |
|---------------|------|------|------|------|------|
| Density Value | 0.28 | 0.37 | 0.45 | 0.46 | 0.43 |
|---------------|------|------|------|------|------|

III. Varying the time of boiling for 150 μg Urea Acid Mixture (C)
Time in minutes 5 10 15 20 25

| | | | | | |
|---------------|------|------|------|------|------|
| Density Value | 0.61 | 1.02 | 1.16 | 1.26 | 1.28 |
|---------------|------|------|------|------|------|

* Unless otherwise stated the test conditions are as described for the standard method

TABLE 2a.

Determination of Fading-Time for Colours developed by the Standard Method.
Acid Mixture (C)

| Time in Minutes | 0 | 20 | 40 | 60 | 120 |
|----------------------------|------|------|------|------|------|
| Density Value (Citrulline) | 0.26 | 0.26 | 0.25 | 0.25 | 0.24 |
| Density Value (Urea) | 0.19 | 0.19 | 0.18 | 0.18 | 0.17 |
| Density Value (Urea) | 1.25 | 1.25 | 1.22 | 1.22 | 1.20 |

APPLICATION OF METHOD TO BLOOD

The method is suitable for routine blood urea analyses. A micro or macro procedure can be adopted depending on the amount of blood available. To precipitate blood protein the tungstic acid method of Folin-Wu should be used. Cadmium or zinc protein precipitates, especially if alkaline, give too low and erratic values. A similar observation was made by Barker (1944). An outline of the procedure for 0.2 ml. blood is now given —

Measure 3.4 ml. of distilled water into a small test-tube, and add 0.2 ml. of blood from a micro-pipette. The pipette is rinsed with the clear supernatant water before laking begins. The tube is shaken and set aside for a few minutes to allow laking to take place, then 0.2 ml. of 10 per cent sodium tungstate solution is added, followed by 0.2 ml. of $\frac{2}{3}$ N sulphuric acid. The tube is shaken again and set aside until the precipitate has turned brown. The contents of the tube are now poured on a small filter-paper (7 cm.). Two ml. of water-clear filtrate are measured accurately into a Folin-Wu tube (graduated at the 9 ml. mark), and in succession 2 ml. of diacetyl reagent and 3 ml. of acid mixture (A) are added. The tube is then filled to the 9 ml. mark with distilled water, inverted to mix all contents thoroughly, and immersed in a boiling water-bath for 20 minutes. After boiling the tube is cooled under running cold water to room temperature. The coloured solution is now poured into one of the Spekker absorptiometer cups. Using both the heat-absorbing filter and the gelatine filter No. 602 in the path of the light, the density value is measured on the instrument.

Calculation With the aid of the standard density values given in the first column of Table 1, a curve is plotted, and the density value of the unknown solution (blood) is referred to this curve to determine its concentration.

The values on the graph read in μg present in 9 ml. volume, and from this the original blood concentration is calculated. Since the 2 ml. of filtrate used correspond to 0.1 ml. of whole blood, the μg value read off on the graph must be multiplied by 1000 to convert it to mg. per 100 ml. of blood. It follows that under these circumstances the value of μg on the graph can be read as mg. per 100 ml. of the blood samples, calculation formulae being thus avoided. If different amounts

of blood are used such a direct reading is not possible and the correct multiplication factors must be used

The accuracy of the method is good when compared with the gasometric urease method. Archibald found the values to be 0.0 to 0.8 mg per 100 ml. higher than those obtained with the urease method. Using urease and the diffusion technique for ammonia estimation (Kawerau, 1941), a few parallel estimations were carried out in this laboratory. The results are offered without comment in Table 3

TABLE 3.

| No. | Specimen | mg urea per 100 ml. | |
|-----|-------------|---------------------|---------------|
| | | Colorimetric Method | Urease Method |
| 1 | Whole blood | 24.0 | 22.0 |
| 2 | Whole blood | 114.0 | 112.0 |
| 3 | Blood serum | 68.0 | 69.5 |
| 4 | Blood serum | 62.2 | 61.5 |
| 5 | Whole blood | 60.0 | 60.0 |
| 6 | Blood serum | 57.0 | 54.8 |
| 7 | Blood serum | 96.0 | 97.6 |

The blood components that might give rise to an "extra-urea" value are citrulline, allantoin, and possibly arginine. Using 30 μ g of each substance the relative interference values were estimated by the standard method. The results are given in Table 4

TABLE 4

| Substance | Density Value of 30 μ g | |
|------------|-----------------------------|----------|
| | Acid (A) | Acid (C) |
| Urea | 0.22 | 0.20 |
| Citrulline | 0.05 | 0.03 |
| Allantoin | 0.01 | 0.02 |
| Arginine | 0.00 | 0.00 |

Archibald (1944) claims that the amount of citrulline in fasting normal human blood is between 0.3 and 1.0 mg per 100 ml of blood, and he gives a similar concentration range for what he terms "apparent allantoin". In using 0.2 ml of blood in the analysis therefore only one third to one tenth of the amount used for the experiments in Table 4 is present, and from the observed density values it can be seen that these substances are not likely to interfere with the accuracy of the method.

APPLICATION OF METHOD TO URINE

To carry out the reaction on urine, the urine must be free from pathological protein. The urine is adjusted to pH 4.5 with 2 drops of 2 per cent acetic acid, boiled and filtered. The filtrate when cold is diluted 1:100. Measure accurately 0.5 ml. of the diluted sample into a Folin-Wu tube and proceed by the method described for blood.

Although acid mixture (A) may be convenient for most workers, in this laboratory for both blood and urine analysis acid mixture (C) is preferred because in the case of urine it is less likely to give rise to extraneous colours. This is probably due to the fact that when finally set up for boiling the acid concentration in the Folin-Wu tube using mixture (C) is only half of that of mixture (A). Archibald (1945) found that with mixture (A) urea values averaged 2.8 per cent higher than the concentrations indicated by the urease method.

APPLICATION OF METHOD TO THE ESTIMATION OF ALLANTOIN AND CITRULLINE.

It is obvious from the foregoing considerations that it is impossible to estimate allantoin and citrulline directly by this method. Special adsorbents for allantoin as well as for citrulline must be used in order to concentrate these substances before the general procedure is carried out. Details have been given by Archibald (1944), and the reader should consult the original paper.

If exactly identical procedures are adopted it has been found possible, in this laboratory, to estimate citrulline by using a methyl urea curve for reference. Gornall and Hunter (1941) point out that the colour intensity is not proportional for the two substances when allowance is made for their different molecular weights. Citrulline is 2.36 times the weight of methyl urea, and if true proportionality existed one could read the μg value of a citrulline pigment on a methyl urea curve and obtain the correct result by multiplying it by 2.36. Since no true proportionality exists on a molecular basis, the factor cannot be used. Still, it must be pointed out that the two curves run parallel to each other, and if the molecular factor does not fulfil the purpose, some empirical factor might be satisfactory. It has been found that the factor of 2 best suits this purpose. A methyl urea curve in which the μg values are multiplied by 2 can serve any citrulline estimation. The error involved in this transaction has been found to be not greater than $\pm 3 \mu\text{g}$ for any single point on the curve.

SUMMARY

A simple direct colorimetric method for the estimation of urea and related carbamido compounds is described. The method is based on the carbamido-diacetyl reaction of Fearon, and has been modified for the purpose of quantitative assay.

The Spekker photoelectric absorptiometer is used to carry out the estimations. A method is described by which it is possible to use methyl urea as a standard for the estimation of citrulline.

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No. 7

THE POSSIBILITY OF INITIATING THERMO-NUCLEAR REACTIONS
UNDER TERRESTRIAL CONDITIONS

By J. H. J. POOLE, Sc.D.

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The recent developments in the generation of energy by uranium fission, have shown that it is possible to generate excessively high temperatures in masses of the order of 1 to 10 kg. It is quite simple to estimate the maximum temperature which could be attained by the complete fission of uranium assuming that all the energy was conserved. The energy per fission is about 177×10^6 electron volts or about 2.8×10^{-4} erg (1). The temperature attained will be so high that we may assume that the fission nuclei are completely stripped of electrons, so that the fission energy will be divided equally between two fission nuclei and 92 electrons, a total of 94 particles. The small number of neutrons emitted may be neglected, as the figure I have quoted is for the energy of the fission products alone, and the total energy per fission is probably of the order of 200×10^6 e.v., so we shall arrive at an underestimate in any case. The mean thermal energy of an elementary particle at a temperature θ is $\frac{3}{2} k \theta$, where $k = 1.38 \times 10^{-16}$ erg per degree; and the energy of each of the 94 particles in the uranium fission is $2.8 \times 10^{-4}/94$ or 3×10^{-6} erg. Hence their temperature is $3 \times 10^{-6}/\frac{3}{2} \times 10^{-16}$ or $15,000 \times 10^6$ degrees. Since at the centre of the sun, whose energy is supposed to be supplied by thermo-nuclear reactions, the temperature is probably of the order of 20×10^6 degrees, it is obvious that the initiation of such reactions by uranium fission is worth considering.

The largest known source of thermo-nuclear energy on the earth is probably the ocean. Solar energy is believed to be generated indirectly by the formation of He^4 from four protons. In this process for every helium nucleus formed 4.2×10^{-5} erg is generated. Now the total mass of hydrogen in the ocean is about 1.6×10^{23} g. (2), and since the number of protons per gram of hydrogen is 6×10^{23} , the total number of protons in the ocean is 9.6×10^{46} . Hence the total energy which could be obtained from the ocean by this method is $\frac{1}{4} [9.6 \times 10^{46} \times 4.2 \times 10^{-5}] = 10^{42}$ ergs.

The amount of hydrogen in the lithosphere would probably only increase this figure by about 10%, and hence need not be considered.

It is of some interest to estimate to what temperature this quantity of energy, if suddenly generated, would raise the temperature of the earth. The mass of the earth is 6×10^{26} g., hence the energy available per gram of earth is $10^{42}/6 \times 10^{26}$

$= 1.67 \times 10^{14}$ ergs. Now Eddington has shown that for a mixture of any set of elements at very high temperatures, the mean atomic weight may be taken as 2, provided no large amount of hydrogen is present. Thus the number of particles between which the 1.67×10^{14} ergs is to be divided will be 3×10^{23} . Hence the energy of each particle will be 5.5×10^{-10} erg, which corresponds to a temperature of $5.5 \times 10^{-10} / 2 \times 10^{-16} = 2.7 \times 10^6$ °C, a temperature which would generate quite a nice little nova!

The theory of thermo-nuclear reactions as applied to stellar conditions has been investigated by Atkinson (3), Gamow and Teller (4), Bethe (5), and others, and their results are equally valid for terrestrial conditions. The equation governing the rate of a thermo-nuclear reaction is according to their work,

$$p = \frac{4 \rho x_1 x_2 \Gamma}{3^{5/2} m_1 m_2 h} \cdot a R^2 \exp 4(2R/a)^{1/2} \cdot \gamma^2 \exp -\gamma$$

where p = number of processes per gram per sec

ρ = density of gas

x_1 and x_2 = the concentrations in grams per gram of mixture of the two reacting nuclear types,

m_1 and m_2 their masses,

R = combined radius

$a = h^2 / m e^2 z_1 z_2$

m = reduced mass $m_1 m_2 / m_1 + m_2$

$z_1 e$ and $z_2 e$ the nuclear charges

Γ/h = the probability of nuclear reaction in sec^{-1} after penetration

$$\gamma = 3 \left[\frac{\pi^2 m e^4 z_1^2 z_2^2}{2 h^2 k \theta} \right]^{1/2}$$

If we measure ρ in g/cm^3 , Γ in electron volts and θ in units of 10^6 °C, Bethe states that

$$\rho = 5.3 \times 10^{25} \rho x_1 x_2 \Gamma \Phi (z_1 z_2) \gamma^2 \exp -\gamma$$

$$\text{where } \gamma = 42.7 (z_1 z_2)^{1/2} \left(\frac{A}{\theta} \right)^{1/2}$$

$$\Phi = \frac{1}{A_1 A_2 (z_1 z_2 A)^3} \cdot \left(\frac{8R}{a} \right)^2 \exp -2 \left(\frac{8R}{a} \right)^{1/2}$$

$$A = m/m_H$$

m_H = mass of hydrogen nucleus

A_1 and A_2 are the chemical atomic weights of reacting types, and for the combined radius R he puts $1.6 \times 10^{-13} (A_1 + A_2)^{1/2}$ cm

In order to get a figure independent of the density and percentage composition of the reacting mixture, he calculates the value of P ,

$$\text{where } P = \frac{m_2}{\rho x_1 x_2} \cdot p$$

Since x_2/m_2 = number of nuclei of type 2 present per gram of mixture

$P \rho x_1$ = probability per sec. that a given nucleus of type 2 undergoes reaction.

If we take a given mixture of two reacting types, i.e. fix ρ , x_1 , and x_2 , then γ is a function of θ only in expression for p , and $\gamma = b\theta^{-1/2}$ where b is a constant.

Calling ε_θ the energy production per g. per sec and Q the energy liberated per process, we see that,

$$\varepsilon_\theta = pQ = B\gamma^2 \exp -\gamma \text{ where } B \text{ is a constant}$$

This gives us the following simple relations,

$$(1) \quad \gamma = b\theta^{-\frac{1}{2}}$$

$$(2) \quad \frac{\gamma_2}{\gamma_1} = \left(\frac{\theta_1}{\theta_2}\right)^3$$

$$(3) \quad \frac{\varepsilon_2}{\varepsilon_1} = \left(\frac{\gamma_2}{\gamma_1}\right)^2 \exp(\gamma_1 - \gamma_2)$$

Hence if γ and ε are known for any temperature, we can easily find them for any other temperature, and also find the value of b

$$\text{Furthermore, since } \varepsilon = B\gamma^2 \exp -\gamma$$

$$\varepsilon \text{ is a maximum when } \gamma = 2.1e \text{ when } \theta = b^3/8$$

In applying these results to the sea, there are four reactions which must be considered. These are proton proton, proton deuteron, and deuteron deuteron reactions, the last of which may take two forms.

The nuclear equations are

$$(1) \quad {}_1H^1 + {}_1H^1 = {}_1H^2 + e^+ + Q_1$$

$$(2) \quad {}_1H^1 + {}_1D^2 = {}_2He^3 + Q_2$$

$$(3) \quad {}_1D^2 + {}_1D^2 = {}_2He^3 + {}_0n^1 + Q_3$$

$$(4) \quad {}_1D^2 + {}_1D^2 = {}_1H^3 + {}_1H^1 + Q_4$$

As far as the deuteron deuteron reactions are concerned, it will be sufficient to consider reaction 3 only, as it constitutes about 60% of the total reaction, and a factor of two in the results is hardly significant

Bethe gives the estimated values of Q , and of γ and P at 20×10^6 °C. His figures are shown in Table I

TABLE I

| Reaction | Q | γ_{20} | P_{20} |
|--|----------|---------------|-----------------------|
| ${}_1H^1 + {}_1H^1 = {}_1H^2 + e^+$ | 1.53 mMU | 12.5 | 8.5×10^{-21} |
| ${}_1H^1 + {}_1D^2 = {}_2He^3$ | 5.9 " | 13.8 | 1.3×10^{-2} |
| ${}_1D^2 + {}_1D^2 = {}_2He^3 + {}_0n^1$ | 3.5 " | 15.7 | 10^3 |

$$1 \text{ mMU} = 1 \text{ milli mass unit} = 0.931 \times 10^6 \text{ electron volts} \\ = 1.49 \times 10^{-6} \text{ erg.}$$

It is to be noted that the values of Γ used in obtaining these results are not of equal certainty. For reaction 1, Γ is calculated from Fermi's Theory of Positron Emission (6), for 2, Γ is apparently only estimated, whereas for 3, Γ is based on actual experimental determinations.

In using these results for terrestrial conditions, the value of ρ to be used is obviously uncertain. It is clear, however, that it cannot exceed the density of the sea in the liquid phase, and accordingly in the following calculations I have taken $\rho = 1 \text{ g./c.c.}$, which will thus probably give a gross over-estimate of the rate of energy production. The concentration of heavy water in the sea is assumed to be 1/5000 that of ordinary water.

To show how the values of energy productions subsequently given have been obtained, I give one example, that of the proton proton collision

Type of Reaction.

$${}_1H^1 + {}_1H^1 = {}_1H^2 + e^+ + Q$$

$$Q = 1.53 \text{ mMU} = 2.3 \times 10^{-6} \text{ erg}$$

$$\gamma \text{ at } 20 \times 10^6 \text{ }^\circ\text{C} = \gamma_{20} = 12.5$$

$$P \text{ at } 20 \times 10^6 \text{ }^\circ\text{C} = P_{20} = 8.5 \times 10^{-21}$$

$$p \text{ at } 20 \times 10^6 \text{ }^\circ\text{C} = p_{20} = P_{20} \rho x_1 x_2 / m_2$$

$$x_1 = x_2 = \frac{1}{5} \text{ nearly}$$

$$\rho = 1 \text{ g./c.c.}$$

$$m_2 = 1.56 \times 10^{-24} \text{ g}$$

$$\text{Hence } p_{20} = P_{20} \frac{1 \times (0.11)^2}{1.67 \times 10^{-24}} = P_{20} \times 7.2 \times 10^{21}$$

$$= 8.5 \times 10^{-21} \times 7.2 \times 10^{21}$$

$$= 61.5$$

Hence the energy liberation per g, per sec

$$\varepsilon_{20} = p_{20} Q = 61.5 \times 2.3 \times 10^{-6} \text{ erg}$$

$$= 1.42 \times 10^{-4} \text{ erg per g per sec.}$$

Since we now have the values of γ and ε at $20 \times 10^6 \text{ }^\circ\text{C}$ we can calculate ε at any temperature, and this has been done for a temperature of $1000 \times 10^6 \text{ }^\circ\text{C}$ and also for the temperature of maximum generation of energy. The results are given in Table 2

TABLE 2.

| Reaction | ε_{20} | ε_{1000} | $\varepsilon \text{ max}$ | $\theta \text{ max}$ |
|--|-----------------------|-----------------------|---------------------------|----------------------|
| ${}_1H^1 + {}_1H^1 = {}_1H^2 + e^+$ | 1.42×10^{-4} | 8.7×10^{-2} | 1.33×10^{-1} | 5270×10^6 |
| ${}_1H^1 + {}_1D^2 = {}_2He^3$ | 8.5×10^{10} | 1.36×10^{14} | 1.90×10^{14} | 6420×10^6 |
| ${}_1D^2 + {}_1D^2 = {}_2He^3 + {}_0n^1$ | 7.5×10^{11} | 6.0×10^{15} | 1.08×10^{16} | 9670×10^6 |

These figures are to some extent surprising, showing that the deuteron deuteron reaction even in ordinary water is the most important one. This is due to the value of P_{20} which, however, is based on experimental determinations, and is thus the most certain value of P_{20} given for the three reactions concerned.

The best way, I think, of applying these results to the question of whether it is possible to initiate and maintain a thermo-nuclear reaction in the sea is to consider a rather artificial case. Supposing we take a sphere of water of density 1 g/cc and radius R in which thermo-nuclear energy is being generated at a rate of ϵ ergs per sec per g. Further let us assume that the sphere radiates as a black body. Then if the temperature is to remain constant; we have

$$\frac{4}{3} \pi R^3 \epsilon = 4 \pi R^2 \sigma \theta^4$$

where σ = Stefans Constant

$$\text{Now } \epsilon = B\gamma^2 \exp -\gamma$$

$$\gamma = b \theta^{-\frac{1}{2}}$$

$$\text{Hence } R = \frac{3 \sigma \theta^4}{B b^2 \theta^{-\frac{1}{2}} \exp -b \theta^{-\frac{1}{2}}}$$

$$= C \theta^{\frac{9}{2}} \exp b \theta^{-\frac{1}{2}}$$

$$\text{where } C = 3 \sigma / B b^2$$

It is easily shown by differentiating that R will be a minimum when $\gamma = 14$. For the deuteron deuteron reaction γ has this value when $\theta = 28 \times 10^6 \text{ }^\circ\text{C}$ which gives the minimum value of R as $3.1 \times 10^6 \text{ km}$.

As this value is so large, there seems to be very little danger of starting a world wide explosion by dropping any possible atomic bomb into the sea. The experiment will probably be made before this paper is published.

It is of some interest to consider the case of pure heavy water. Table 3 gives the relevant figures.

TABLE 3.

| Reaction | ϵ_{20} | ϵ_{1000} | $\epsilon \text{ max}$ | $\theta \text{ max}$ |
|------------------------------|----------------------|-----------------------|------------------------|----------------------|
| $1D^2 + 1D^2 = 2He^3 + 0n^1$ | 6.8×10^{19} | 5.45×10^{23} | 9.8×10^{23} | 9670×10^6 |

From these it can be shown that in this case the minimum radius of our hypothetical sphere would be only 3.6 km., so that even in an ocean of heavy water it would seem unlikely that a self-maintaining thermo-nuclear explosion could be started with an atomic bomb. It does seem probable, however, that if heavy water is incorporated in the bomb, some of the energy liberated in the explosion will be derived from a deuteron deuteron reaction, and that the neutrons emitted by the reaction would aid in ensuring the more complete fission of the active heavy element constituent.

In conclusion it may be pointed out that, although the method used in determining the critical radius of a self-maintaining thermo-nuclear explosion is physically very crude, it seems unlikely that any more complete investigation would lead to a large decrease in the critical radius, since in any actual explosion there will be a rapid decrease in density of the reacting components which would reduce the energy output. It is unlikely also that the assumption of black body radiation would be so far from the truth as to invalidate the conclusion that a self-maintaining thermo-nuclear reaction cannot be started in the sea by uranium fission.

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No. 8.

THE MECHANISM OF THE FORMATION OF ALLOPHANATES FROM
CARBAMATES

By

A. E. A. WERNER AND J. J. GRAY.

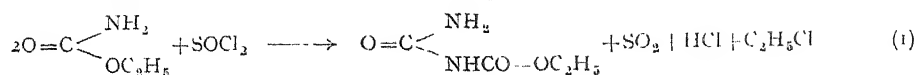
[Read DECEMBER 18, 1945. Published separately MAY 6, 1946].

SINCE the discovery of ethyl allophanate by Liebig (1) numerous methods have been described for its preparation. These methods have been conveniently summarised by Bougault and Leboucq (2). Although the conversion of urethane into ethyl allophanate can be carried out by such reagents as sulphuryl chloride (3), phosgene (4), and phosphorus pentoxide (5), with varying yields, chief interest centres on the use of thionyl chloride. Schroeter and Lewinski (6) obtained ethyl allophanate by refluxing urethane with thionyl chloride in benzene solution, and later Warren and Wilson (7) repeated the reaction using a large excess of thionyl chloride in the absence of a solvent. In a recent communication one of us (A. E. A. W. 8) confirmed the work of Warren and Wilson but showed that it is not necessary to use a large excess of thionyl chloride, since urethane can be quantitatively converted into ethyl allophanate by warming it at 60° C. for 1½ hours with just one molecular proportion of thionyl chloride. Very shortly after the publication of this communication Raiford and Freyermuth (9) confirmed the findings of Schroeter and Lewinski but reported that they were unable to obtain any ethyl allophanate according to the method of Wilson and Warren, obtaining instead only a small amount of cyanuric acid. In view of our results it is very difficult to understand the failure of Raiford and Freyermuth to obtain ethyl allophanate using Warren and Wilson's technique. Unfortunately they have omitted to give any experimental details, the only explanation which suggests itself is the possibility that overheating may have occurred, since it has been shown (A. E. A. W. 8) that on heating ethyl allophanate is readily decomposed into cyanuric acid.

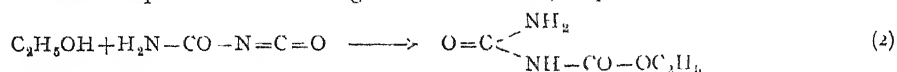
Since the reaction of Warren and Wilson as modified by us is of practical importance because it constitutes the best method for the preparation of ethyl allophanate, considerable interest also lies in the elucidation of the actual mechanism by which the reaction proceeds. Previous workers, including also Warren and Wilson (*loc. cit.*), without offering any explanation of the actual mechanism, contented themselves with the statement that the thionyl chloride acted as a de-

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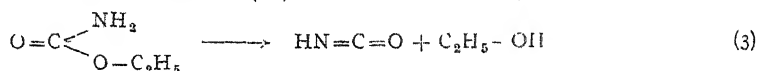
alcoholating agent, removing one molecule of ethyl alcohol from two molecules of urethane in accordance with the following equation --



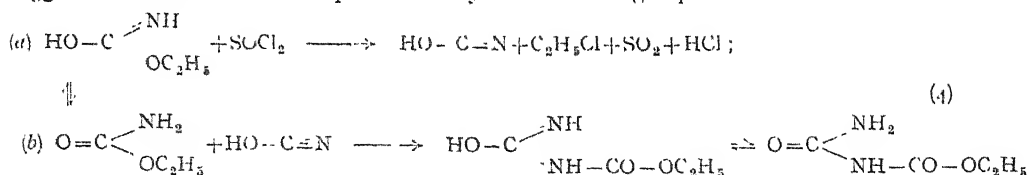
Davis and Blanchard (10), in a communication on the hypothetical substance dicyanic acid, suggest that alcohols are capable of uniting directly with this dicyanic acid to form allophanates according to the following equation --



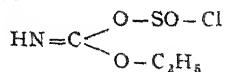
Although the experimental conditions would not be favourable to the formation of a substance apparently so unstable as dicyanic acid, the possibility might still remain that the thionyl chloride functions by accelerating the decomposition of the urethane molecule at a lower temperature in a manner similar to its thermal decomposition which, as E. A. Werner (11) has shown, takes place slowly thus --



The cyanic acid thus formed might, according to the hypothesis of Davis and Blanchard, dimerise and combine with alcohol to give ethyl allophanate. Since the existence of the substance dicyanic acid must be regarded as very doubtful,¹ an alternative mechanism was put forward in our original communication in which it was tentatively suggested that the formation of ethyl allophanate proceeds in two stages. In the first stage it was assumed that cyanic acid was liberated in an "active" form from one molecule of urethane, followed in the second stage by the addition of this "active" cyanic acid molecule to unchanged urethane. This suggested mechanism was represented by the following equations --



which proceed simultaneously after the initial decomposition of the urethane has started. Raiford and Freyermuth (*loc. cit.*) incidentally suggested a similar mechanism and showed that the thionyl chloride initially reacted with urethane to produce an intermediate complex of the type



which then decomposed into sulphur dioxide, ethyl chloride, and cyanic acid, which was assumed to combine with unchanged urethane to give ethyl allophanate.

It is well known that cyanic acid readily combines with alcohols in neutral solution forming carbamates, or with amines forming substituted ureas; there is, however, no direct experimental evidence to show that cyanic acid is normally

¹ We have repeated the experiments described by Davis and Blanchard to show the existence of a solution of dicyanic acid but have been unable to confirm their results. This work will be the subject of a forthcoming communication.

capable of reacting with the carbonamide group, as postulated in the second stage of the mechanism suggested in our original communication. It was for this reason that an 'active' form of cyanic acid was originally suggested as being necessary. The present communication is therefore concerned with the presentation of additional experimental evidence to support the suggested mechanism, and to show that under suitably chosen conditions cyanic acid is liberated in an activated form, which readily reacts with the carbonamide group.

A series of experiments was carried out in which cyanic acid was generated by different methods and allowed to react directly with urethane under different experimental conditions, so chosen that they would permit of an unambiguous interpretation. Under certain conditions it was found that no ethyl allophanate was formed, but if the correct experimental conditions were chosen direct addition of cyanic acid to the carbonamide group of the urethane molecule occurred, leading to the formation of ethyl allophanate in good yield. The actual techniques employed may be conveniently discussed under the following headings.—

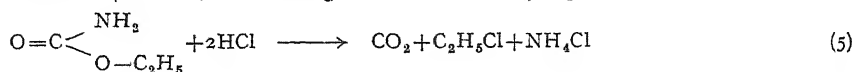
(i) *Reaction between cyanic acid gas and urethane in neutral media.* Hofmann (12) claimed that ethyl allophanate was formed by passing cyanic acid vapour directly into molten urethane, but he himself did not give any experimental evidence in support of his statement. Davis and Blanchard (*loc. cit.*) state that Béhal (13) failed to confirm Hofmann's result, but this is incorrect, since Béhal does not mention having repeated Hofmann's work, and in point of fact a survey of the chemical literature has shown that Hofmann's claim has never been experimentally tested. According to our experimental results, however, no trace of ethyl allophanate was produced when cyanic acid gas was passed into molten urethane. In each case the sole products of the reaction were cyanuric acid and unchanged urethane. A similar result was obtained when an ethereal solution of cyanic acid was added to an ethereal solution of urethane at room temperature, after a few days the only insoluble product that settled out was cyanuric acid. From these results it may be reasonably concluded that in neutral media cyanic acid does *not* react with urethane.

(ii) *Cyanic acid generated in glacial acetic acid solution.* Bailey and his co-workers (14) have shown that, in the case of a number of weak nitrogen bases, carbamide derivatives are readily formed in glacial acetic solution by stirring in finely ground potassium cyanate, and they have demonstrated the usefulness of this modified form of the Wohler synthesis in many cases. This technique was applied by us, but the expected addition reaction between urethane and cyanic acid did not take place, only ill-defined polymerisation products were obtained, and no trace of ethyl allophanate could be detected.

(iii) *Cyanic acid generated by the thermal decomposition of urea.* Since ethyl allophanate is produced in large yield by the action of thionyl chloride on an equimolecular mixture of urea and urethane, as shown in our original communication, an experiment was now carried out in which an equimolecular mixture of urea and urethane was heated at a temperature of 145°–170° C for a few hours. The result of this experiment was, however, in direct contrast to that in which thionyl chloride was used. No trace of ethyl allophanate could be detected among the reaction products, which were found to consist of unchanged urethane, biuret, and cyanuric acid. This result is in agreement with the work of Davis and Blanchard (*loc. cit.*),

who failed to obtain *n*-butyl allophanate when they heated *n*-butyl carbamate with urea or nitrourea. Their interpretation of this result as proving that allophanates are not formed by addition of cyanic acid to carbamates, but are formed directly from dicyanic acid and an alcohol is however incorrect. The true explanation of the non-formation of ethyl allophanate is given later in the text.

In contrast to the above experimental results it was found that ethyl allophanate was readily produced when cyanic acid was generated in the presence of hydrochloric acid. In the first series of experiments *dry* hydrochloric acid gas was passed into a suspension of potassium cyanate in molten urethane, and ethyl allophanate was produced in good yields. The yield decreased with rise in temperature, and at 140° C amounted to only 7% of the theoretical. This decrease in the yield of ethyl allophanate with increase in temperature is due to the fact that at the higher temperature the urethane is largely decomposed by the hydrochloric acid, as shown by E. A. Werner (*loc cit*), according to the following equation:—



Our experimental results confirm this conclusion. At 50° C. there is no appreciable decomposition when hydrochloric acid gas is passed into molten urethane, at 80° C. decomposition is appreciable, and from the weight of ammonium chloride formed it is possible to calculate the amount of urethane decomposed in this manner, and finally at 140° C decomposition is so rapid that a copious precipitate of ammonium chloride forms immediately. Since no cyanuric acid is produced in these experiments it is clear that thermal decomposition of urethane according to equation (3) does not occur. This disposes of any possibility that the ethyl allophanate could be formed by the mechanism favoured by Davis and Blanchard (*loc cit.*). Furthermore, if the formation of ethyl allophanate were connected with the decomposition of the urethane molecule, the yield of ethyl allophanate should be zero at 50° C. and a maximum at 140° C. In point of fact the experimental findings are quite the reverse, thus indicating that the ethyl allophanate is actually formed by the direct union of cyanic acid with the carbonamide group of the urethane molecule.

This conclusion received further experimental support from a final series of experiments in which cyanates of potassium, sodium, or silver were added to a solution of urethane in *anhydrous* ether which had been saturated with *dry* hydrochloric acid gas. After the reaction mixtures had stood at room temperature for a few hours a crystalline precipitate of ethyl allophanate was collected, the yield amounting to about 70% of the theoretical. The reaction also proceeded satisfactorily using anhydrous benzene or anhydrous dioxane as solvents. This technique is so simple and gives such satisfactory results that it was extended to the synthesis of a whole series of allophanates from the corresponding carbamates as described in the experimental part.

This final series of experiments affords conclusive evidence that it is possible to bring about the direct addition of cyanic acid to the carbonamide group $-\text{CONH}_2$, if the cyanic acid is liberated in the presence of hydrochloric acid. An active complex, namely nascent carbamyl chloride, is formed '*in situ*' according to the following equation:—

EXPERIMENTAL.

Reaction between cyanic acid gas and urethane (i) *In molten state* Cyanuric acid (1.5 g.), previously dehydrated by drying at 110° C., was heated to dull red heat in a hard glass tube in a tubular electric furnace. The cyanic acid vapour was passed into urethane (5 g.) contained in a U-tube maintained at a temperature of 55° C. An opalescence developed in the molten urethane, and after extraction with ether an insoluble residue remained which was identified as cyanuric acid by the usual tests. A similar result was obtained when the reaction was repeated at 80° C. and 140° C.

(ii) *In ethereal solution.* To four tubes containing an ethereal solution of cyanic acid (0.6 g.) urethane (1 g., 2 g., 3 g., and 5 g.) was added. After standing a few days at room temperature a white precipitate settled out which was identified as cyanuric acid.

Reaction between cyanic acid and urethane in glacial acetic acid. To a solution of urethane (8.9 g.) in glacial acetic acid (30 c.c.) potassium cyanate (8.1 g.) was slowly added. The resultant semi-solid mass was warmed on the water-bath for one hour. Subsequent treatment with ether to remove unchanged urethane caused the precipitation of a crystalline substance, M.P. 110° C., identified as a molecular compound of acetic acid and potassium acetate (cf. Beilstein vol. II, p. 108). After extraction with distilled water a residue of ill-defined polymerisation products of cyanic acid remained from which boiling benzene failed to extract any ethyl allophanate.

Action of heat on an equimolecular mixture of urea and urethane An intimate mixture of urea (9 g.) and urethane (14 g.) was heated in an oil-bath. A homogeneous melt formed at 95° C.; reaction started at 160°–170° C. with copious evolution of ammonia, ethyl alcohol distilled over and a white solid separated. The heating was continued for three hours. Ethereal extraction removed unchanged urethane (0.5 g.) leaving a solid residue (13.5 g.), which was treated with a volume of caustic soda solution just sufficient to cause complete solution. Passage of carbon dioxide through this solution precipitated biuret in needle-like crystals (5 g.). Further acidification with dilute acid precipitated cyanuric acid (8 g.). No ethyl allophanate could be detected.

Reaction between cyanic acid and urethane in presence of hydrochloric acid (i). *In molten state.* Dry finely powdered sodium cyanate (10.6 g.) was suspended in molten urethane (15 g.) maintained at 80° C., and dry hydrochloric acid gas was passed in for 30 minutes. When cool the contents of the flask were extracted with ether to remove unchanged urethane (10.9 g.). The residue was treated with cold water (75 c.c.) and filtered. The amount of undecomposed sodium cyanate in the filtrate was determined and found to be 5.28 g. The remaining insoluble residue was dissolved in hot water, and titrated while still hot with N sodium hydroxide, converting any cyanuric acid present into soluble sodium hydrogen cyanurate. On cooling a white microcrystalline precipitate settled out. Weight, 3.9 g. M.P. 193° C., not depressed by mixture with an authentic specimen of ethyl allophanate. Upon acidification cyanuric acid (1.6 g.) was obtained. From the above data it follows that the amount of cyanic acid actually liberated was 3.55 g., of which 1.6 g.

polymerised to cyanuric acid, leaving 1.95 g. available for union with urethane to give ethyl allophanate (theoretical yield=5.99 g). Hence actual yield of ethyl allophanate is 65% of the theoretical. The formation of this amount of ethyl allophanate would require 2.63 g of urethane. Hence weight of urethane directly decomposed by the action of the hydrochloric acid was 1.47 g.

(ii) *In ether or dioxane solution* (a) Urethane (8.9 g) was dissolved in anhydrous ether (50 c.c) saturated with dry hydrochloric acid gas ($\text{HCl}=\text{ca. } 3\text{N}$) contained in a conical flask closed with a calcium chloride tube. Dry finely powdered sodium cyanate (6.5 g) was added slowly in small amounts. After the mixture had stood overnight, the supernatant ethereal layer was decanted and the solid residue drained on the filter-pump. After extraction with cold water (50 c.c) to remove sodium chloride an insoluble residue remained which melted at 193°C , and showed no depression on mixing with an authentic specimen of ethyl allophanate. Weight, 7.9 g., corresponding to 67% of the theoretical.

(b) To a solution of urethane (4.5 g) in anhydrous dioxane saturated with hydrochloric acid ($\text{HCl}=5\text{N}$) silver cyanate (7.5 g) was added slowly. After standing overnight the dioxane layer was decanted, and the silver chloride residue extracted twice with hot water. The hot filtrate deposited microcrystalline ethyl allophanate, M.P. 193°C . Weight, 3.7 g., corresponding to 56% of the theoretical.

Decomposition of urethane by hydrochloric acid Dry hydrochloric acid was passed for 30 minutes into molten urethane (5 g) contained in a wide-necked flask maintained at the following temperatures—(i) At 50°C . No reaction occurred, the contents of the flask were completely soluble in ether. (ii) At 80°C . A slight reaction occurred, and the residue left after extraction with ether consisted of pure ammonium chloride. Weight, 0.82 g. Tests for the presence of ammonium cyanate or cyanuric acid were negative. (iii) At 140°C . A copious precipitate of ammonium chloride formed immediately. The weight, after removal of unchanged urethane, was 1.9 g, corresponding to 63% of the theoretical. Even at this elevated temperature no trace of ammonium cyanate or cyanuric acid could be detected.

Preparation of allophanates from the corresponding carbamates In each case the preparation was carried out by adding an equimolecular proportion of sodium cyanate to an equimolecular proportion of the carbamate dissolved in ether or dioxane saturated with dry hydrochloric acid. Unchanged carbamate was removed by extracting the solid residue with cold alcohol, and the allophanate was purified by recrystallisation from hot water or alcohol. In this manner the following allophanates were prepared in excellent yields, and identified by their M.P.s which agree with those given in the literature. Methyl M.P. 210°C . *n*-Propyl M.P. 175°C .; *iso*-Propyl M.P. 179°C .; *n*-Butyl M.P. 149°C . and *iso*-Amyl M.P. 160°C . The successful outcome of these preparations necessitates the use of anhydrous reagents and the complete exclusion of moisture, in order to prevent hydrolysis of the carbamyl chloride to ammonium chloride and carbon dioxide.

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No. 9.

DOPPLERITE IN CO. LIMERICK.

M. DEE and G. F. MITCHELL.

[Read NOVEMBER 27, 1945. Published separately MAY 7, 1946].

WHILE engaged in superintending the production of turf in Co. Limerick in 1944 one of the authors (M.D.) noticed that the turf being used in a forge at Lisnagry contained a black pitchy-looking substance that burnt very vigorously. Enquiries revealed that the turf came from a cutting in a nearby bog in Mountshannon Townland (6" O.S. Map Limerick Sheet 6 ; 22.5 cm. from top, 33.5 cm. from left) where fresh dopplerite could be seen in a vein-like mass intersecting the peat face. The authors made a detailed examination of the site in August, 1945. As previous records of the occurrence of this mineral in Ireland are few in number the discovery seemed worthy of record. In 1903 Moss (1) recorded dopplerite from a bog in Co. Antrim, and there are two records (2 & 3) prior to 1850 (when the mineral was first described and named) which certainly refer to dopplerite.

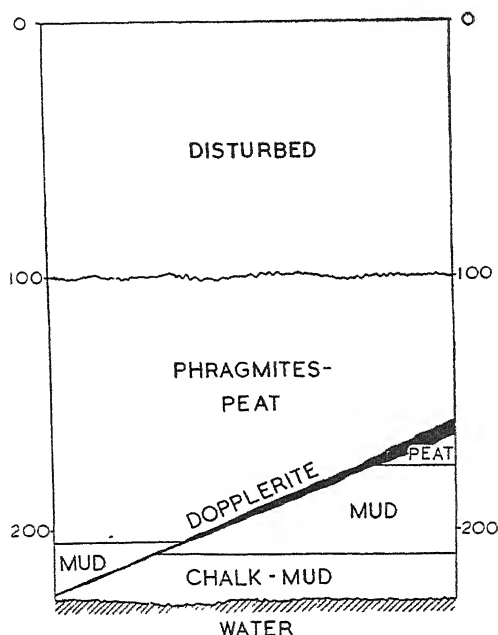
In the area north-east of Limerick city there are large expanses of raised-bog, which have been extensively cut away in the vicinity of Lisnagry. That the cut-away bog in Mountshannon Townland was originally a raised-bog was shown by a surviving block of isolated peat, which had the typical stratigraphy :—

- Fresh *Sphagnum*-peat.
- Highly-humified *Sphagnum*-peat.
- Phragmites*-peat rich in wood (Marsh-peat).
- Mud (laid down in open water).
- Chalk-mud (laid down in open water).
- Sandy clay (of glacial origin).

In the immediate vicinity of the cutting showing the dopplerite all the fresh *Sphagnum*-peat and most of the highly-humified *Sphagnum*-peat had been cut away. The existing cuttings (in *Phragmites*-peat) go below the local water-table and fill with water as soon as cutting ceases. When as much water as possible had been pumped out the section illustrated was seen in a corner of the cutting.

The upper metre of peat was disturbed material from former cuttings, and underneath this was *Phragmites*-peat. It was clear that the upper part of the

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Section in turf-cutting, showing dopplerite lying above a fault in the turf.
Depths in centimetres.

Phragmites-peat in the section had been faulted downwards and towards the north in relation to the lower part of the section, the vertical movement being about 30 cm. Due to the disturbance of the surface it was not possible to trace the fault beyond the limits of the cutting which was 6 m long. There were deep cuttings (now abandoned) to the north of the cutting, and the fault had presumably developed after these cuttings removed support from the north side of the remaining peat. The effect of the fault was to bring the *Phragmites*-peat which was stratigraphically above the mud down into lateral contact with the mud.

The dopplerite had developed in the plane along which movement had taken place and so occurred in a vein-like mass. It had developed most freely (to a thickness of 6 cm) where peat remained in contact with peat across the fault, it was less thick where peat had been faulted against mud, and only appeared as a thin streak where mud remained in contact with mud across the fault. In some places patches of apparently unaltered peat were completely surrounded by dopplerite; in others, where stems of *Phragmites* still surrounded by peat abutted against the main mass of the dopplerite, the cavity in the stem for some little distance away from the contact was found to be filled with dopplerite.

These observations suggested that the dopplerite was of recent formation, being subsequent to the earlier cuttings and the development of the fault. Its presence where peat abutted against the fault and its absence where mud only abutted against the fault suggested that the dopplerite had been formed from the constituents of the peat. The plane of the fault would allow of freer movement of

gases and liquids than would be normally possible in peat or mud, and such a circulation may have assisted the formation of the dopplerite. In a laboratory experiment the introduction of some uncontaminated dopplerite into an agar plate was followed by a growth of bacterial colonies and a marked liquifaction of the agar in the vicinity of the colonies. It is possible that in the alteration of peat to dopplerite bacterial action may play a part.

Schreiber (4) in his book *Moorkunde** discusses the occurrence of dopplerite in bogs on the continent. He states that it occurs most frequently in gaps, veins, and fissures, and in the vicinity of pieces of wood and plant stems, especially filling up cavities in the latter. He explains the origin of the fissures as usually caused by a shrinking of the bog due to a dry climatic phase, the fissure being later filled with peat when the bog growth was resumed. He makes no reference to the formation of fissures by faulting in the peat. He considers that the dopplerite is often in a secondary position, as it is sometimes found, not only in the peat itself, but also in the sub-soil underneath the bog. One of the early Irish records (3) describes 'a substance externally resembling bitumen' 'of a jetty black colour—soft and spongy when first removed from the gravel, with a conchoidal fracture' (almost certainly dopplerite) in the gravel below a bog in Co. Kildare. Schreiber pictures the cell-walls of the plant-tissues decaying into dopplerite, and the mineral being carried by water into suitable resting-places, though he does not deny the possibility that it may sometimes occur *in situ*. Abroad, as at Lisnagry, it occurs most frequently in *Phragmites* peat but has also been found in peat built up of moss remains. The position of the dopplerite at Lisnagry makes it almost certain that it must have been derived from the *Phragmites* peat and not from some other peat from whence it had migrated into its present position. The fault (along the plane of which it lies) could not have developed until extensive cutting had removed upper layers of peat leaving only *Phragmites* peat *in situ*. Also the nuggets of apparently unaltered peat entirely surrounded by dopplerite suggest that a progressive alteration was moving out from the plane of the fault. Dopplerite was scarce wherever mud was in contact with mud. This mud, formed chiefly of the *excreta* of the *plankton* that lived in the open water of the lake that preceded the marsh, is poor in cellulose, and thus may explain the paucity of dopplerite in its vicinity. The calcium carbonate in the underlying chalk-mud was probably deposited by *Chama*, but this chalk-mud was also poor in cellulose.

Two of the earlier Irish records (1 & 3) place the dopplerite at or below the bottom of bogs that had been disturbed by human activities. This seems to be the typical location on the continent also, and it is possible that dopplerite may form where an alteration in the natural conditions allows gases and liquids to percolate more freely. This circulation, perhaps assisted by bacteria, brings about the transformation of plant debris to amorphous jelly. Dopplerite is therefore of recent origin, and cannot be regarded as a substance of high antiquity, or as an intermediate stage in the transformation of peat to coal.

*The authors are indebted to Dr J. Lennon of the Industrial Research Council for drawing their attention to this reference.

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No. 10.

OBSERVATIONS ON THE PASMO DISEASE OF FLAX AND ON THE
CAUSAL FUNGUS *SPHAERELLA LINORUM* WOLLENWEBER.

BY

J. B. LOUGHNANE AND R. MCKAY, Department of Plant Pathology, University
College, Dublin;

AND

H. A. LAFFERTY, Seed Testing Station, Dublin.

(PLATES 2, 3 AND 4).

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INTRODUCTION.

PASMO is a Spanish word meaning spasm, and is the common name given to a disease of flax which was first described by Spegazzini (12) in the Argentine in 1911, the causal fungus being then named *Phlyctaena linicola*. The disease was afterwards recorded and described by Brentzel (2) in the U.S.A. The first record of the disease in the old world is apparently that by Rost (11) who in 1936 reported it as causing heavy damage to flax in Yugoslavia. He named the fungus *Septoria linicola*. Wollenweber (15) in 1938 found and described the perithecial stage of the parasite responsible for pasmo, and as a result changed the name of the fungus to *Sphaerella linorum*. During the last ten years the disease has spread extensively throughout many of the flax-growing regions of the world, being reported from Germany (14), Denmark (1), Hungary (5), Russia (8), Kenya (9), and New Zealand (4). Colhoun and Muskett (3) worked with pasmo-infected material received from Kenya, but beyond identifying the organism and proving its pathogenicity under greenhouse conditions no detailed investigations of the disease was made under the circumstances.

The first occurrence of Pasmo in Ireland is that reported by Lafferty and McKay (7) in 1944. They found a fungus growing on wild flax, *Linum angustifolium*, on a farm in the South of Ireland. Morphologically this fungus corresponded with published descriptions of *S. linorum*, and cross inoculations by means of spore suspensions showed that the fungus was capable of causing pasmo symptoms on cultivated flax, thus leaving little doubt as to the identity of the organism.

Following this report a survey of flax diseases was undertaken in the twenty-six counties in 1945—a report of which will appear elsewhere—and at the same time a study of pasmo on flax was carried out at the Albert Agricultural College, Glasnevin. The latter study together with an account of the known distribution of the disease in Ireland constitute the subject of the present paper.

INVESTIGATION OF THE DISEASE AT GLASNEVIN

Symptoms on Seedlings—In October, 1944, pycnosporos derived from a single spore culture of *S. linorum* originally isolated from *L. angustifolium* were suspended in sterile water and sprayed by means of an atomiser on to flax seedlings of the variety Liral Monarch growing in pots of sterile soil in a glasshouse. The pots were covered with belljars for ten days after inoculation so that a very moist atmosphere was maintained around the seedlings. Similar pots of seedlings sprayed with sterile water were kept under the same conditions and acted as controls. The first symptoms appeared on the inoculated seedlings at the end of eleven days as minute circular pale spots on the cotyledons. These lesions enlarged rapidly and developed a water-soaked appearance, later becoming grey or silvery in colour and somewhat irregular in shape (Fig. 1, Plate 2). Sixteen days after inoculation whole cotyledons were withered and hanging down on the plants, and after a further five days abundant black pycnidia developed upon them (Fig. 2, Plate 2). These pycnidia gave rise to the characteristic pycnosporos of *S. linorum*. There was no evidence that the fungus had spread upwards along the plants and five months after inoculation there was no trace of the disease beyond diseased areas on the stems below the point of attachment of the cotyledons. This, however, is not surprising, as under the conditions existing in the glasshouse there was no means by which the spores could have been disseminated. Furthermore, it was later found, and the fact has been commented upon by other workers on this subject, that flax plants between the cotyledonary and flowering stages are very resistant to infection by *S. linorum*.

Confirmation of the temporary resistance of flax to infection by this parasite was obtained from observation on crops which developed from naturally infected seedlings. The first flax seedlings with pasmo disease came to hand early in May, 1945. The attack was quite severe, resembling that of Seedling Blight caused by *Colletotrichum linicolum*. An examination of the crop from which these seedlings came was carried out in the month of June. At this time the presence of the disease could not be verified by field inspection, and it was only when suspicious-looking plants were brought to the laboratory and examined microscopically that the presence of the disease was confirmed. Moreover in the case of a crop of oil flax of the variety Redwing the disease did not attract any attention until September, when the crop was almost mature.

SYMPTOMS ON MATURING PLANTS.

On flax plants which have reached the stage of boll formation, the most striking symptom is a pronounced blight (Fig. 3, Plate 2). This is brought about by the destruction of the leaves on which numerous lesions develop. These diseased areas

are at first somewhat circular in shape, of a brownish grey colour, sometimes with a lighter centre, and are surrounded by a narrow band of chlorotic tissue. Frequently the lesions enlarge in size so that they coalesce, and the whole leaf is rapidly killed. In the final stage the leaf lesions become bleached, especially towards the centre, and numerous black pycnidia develop upon them (Fig. 4, Plate 2). These pycnidia are easily seen against the light-coloured background of the diseased tissue. On the stems infection occurs at any point, and the diseased areas are elliptical in shape, rarely completely surrounding the entire stem, and vary in length from one quarter to one inch (Fig. 5, Plate 2). In colour they are at first dark red or purple, later becoming brownish and sometimes tending to bleach out to a light colour. Pycnidia of the fungus develop also on the stem lesions, and under very moist conditions tendrils of pycnosporangia exude from them. These spore tendrils are horn-like or coiled, and when they are numerous they give a hairy appearance to the diseased areas (Fig. 8, Plate 3). Similar symptoms occur on the flowering branches. The sepals are extremely susceptible to infection, and when diseased they at first turn brown. Later the colour becomes light or silvery, and pycnidia develop profusely upon them (Fig. 6, Plate 3). This bleaching of the sepals with the pycnidial development is a rather striking symptom of the disease. The sepals appear to become entirely permeated by the parasite, and from them the pedicel at its junction with the boll is invaded. This invasion of the pedicel is significant, as will be seen later.

In the final stages of the disease, the leaves fall off, and the bare stems present a mottled appearance compounded of the light green of the healthy portions of the stems and the darker colour of the lesions. In the case of plants which became attacked about flowering time the bolls remain small in size, and the seed from such bolls is somewhat shrivelled, light, and discoloured.

Under favourable conditions the disease spreads from plant to plant with great rapidity. Such favourable conditions include high atmospheric humidity or heavy rainfall occurring at the susceptible stage in the growth of the host plant. Spread of the parasite under these conditions seems to be brought about mainly by wind-borne spores, although rain splashes or insects would no doubt play a part in transferring spores from diseased to neighbouring healthy plants.

The Fungus.

The source of the fungus in the present investigations was a wild flax plant from which Lafferty and McKay had recorded the disease. A pure culture of the parasite originating from a single spore was obtained by the dilution method, and this culture or sub-cultures from it were used throughout the work. The fungus grows readily on potato glucose agar, the medium on which the growth of the fungus is described. The rate of growth is slow, colonies reaching a diameter of 1.50 cm, when incubated for 10 days at 21°C. Such colonies are roughly circular in shape with an entire or very slightly lobed or wavy margin. The centre of each colony is almost black, the texture of the medium is somewhat tough, and the surface is overgrown with whitish aerial mycelium. Cream to orange coloured beads of pycnosporangia occur towards the centre of each colony (Fig. 14, Plate 4), and these

arise from pycnidia which are submerged in the medium. When colonies are crowded upon a plate they remain small, usually not exceeding 0.5 cm in diameter. A few pycnidia are produced in such colonies.

The pycnidia of *S. linorum* are almost black in colour with a definite wall, and occur submerged in the culture medium or in the host tissue. They open through a round ostiole which when complete is 30–45 μ in diameter. The pycnidia vary in diameter from 60 μ up to 135 μ and average 95 μ .

The pycnosporos (Fig. 11, Plate 3) are curved, straight, or slightly bent, with rounded ends. They are hyaline and are generally 3-septate although individuals have been noted with up to five septa. The size of the pycnosporos varies, the average being 26 $\mu \times 2.8 \mu$ and the extremes 21 $\mu \times 2.5 \mu$ and 33 $\mu \times 3.3 \mu$. Pycnosporos from a flax stem collected in Co. Cork averaged 31 $\mu \times 4.1 \mu$ the extreme limits being 30 $\mu \times 4 \mu$ and 35 $\mu \times 4.5 \mu$.

Pycnosporos germinate readily in water or on slides in a saturated atmosphere, and after 24 hours at room temperature produce germ tubes 20 μ to 40 μ long. These in the majority of cases arise from the terminal cells, but they may arise from any cell. A rather striking feature of the germinated pycnosporos is their power to conjugate or anastomose (Fig 12, Plate 4), but the significance of this is not understood. Another type of germination was observed where the pycnosporos budded off an elliptical secondary spore about 10 μ in length, which having become detached germinated in turn by the production of a thin germ tube from either or both ends.

Brentzel (2) in the U.S.A. has described and figured from artificial cultures of *S. linorum* bodies resembling chlamydospores, and found that they were capable of germinating under suitable conditions. The present authors have found bodies of a similar nature occurring amongst pycnosporos not alone from old cultures, but also from diseased stems, seeds and boll tissue. These chlamydospores are generally rounded in shape, wider than the normal width of the cells of the pycnosporos which give rise to them, and with slightly thicker walls than the latter (Fig. 13, Plate 4). Frequently the contents of an adjoining cell appear to be absorbed in the formation of a chlamydospore, but pycnosporos have been observed where each of the four cells developed into a chlamydospore. The chlamydospores in turn become divided by a cross wall, so that each forms two separate spores. They germinate in the same manner as pycnosporos, but the germ tubes arising from them are generally stouter than those from the latter. Chlamydospores are said to be resistant to conditions which may be adverse to the fungus, and may thus be a means of ensuring the persistence of the parasite during the winter or unfavourable periods—such as prolonged drought.

Dissemination of the Disease by the Seed.

Most workers on the subject have reached the conclusion that pasmo disease of flax is seed borne. Thus Brentzel (2) found pycnosporos of *S. linorum* in washings of seed from an infected crop, and as a result he concluded that the parasite is carried as a contamination on the surface of the seed. Wollenweber (15), advancing as evidence the observation that in pasmo pycnidia occur on the seed capsules, concluded that the fungus is carried on the seed. Natrass (9), on the

other hand, failed to find *S. linorum* in centrifuged washings of seed from a diseased crop, but nevertheless considers it safest to assume that the fungus is seed-borne, and believes that small particles of infected material may carry pycnidia and dried spore masses. The present authors have found that, when seed was separated from crushed infected bolls and shaken up in a small quantity of water which was later centrifuged, the sediment contained numerous pycnospores of *S. linorum*. It has never, so far as the authors are aware, been proved experimentally that such contaminated seed could give rise to the pasmo disease, and it is rather significant that the majority of workers on this disease refer to the great difficulty of controlling it by seed treatment. Amongst these may be mentioned Rost (11), Stapel (13), and Newhook (10). Stapel points out that the fungicidal action of the plant-protectives rose as the quantities were increased, but a very adverse influence was exerted on germination and growth of the seed, and such treatments are not practicable on a large scale. Newhook in tests carried out in New Zealand with hot water and four dusts found that only the former gave any measure of control, immersion of the seed for ten minutes being necessary at a temperature of 127°F.

While pasmo may be spread by detritus amongst the seed or by spores adhering to the surface of the seed these are not the only methods of seed dissemination, nor are they the most important ones, as will now be shown.

Seed Infection.

A careful examination of numerous bolls from infected flax plants was made, but on none was there any indication that the pericarp or wall of the boll was invaded by the parasite. This fact led to the conclusion that if seed became infected it could do so only by invasion from the placenta through the funicle. As already stated, in the case of flax plants affected by pasmo the sepals become permeated by the fungus. It was later observed that in almost all cases where this occurred the parasite made its way from the diseased sepals to the pedicel at its junction with the boll. When the bolls borne on such diseased pedicels were broken open many of them had pycnidia developed in profusion along the placenta and on the dissepiment (Figs. 15 & 17, Plate 4). Furthermore, many of these pycnidia had produced extremely long spore tendrils (Fig. 7, Plate 3). These tendrils at the time of examination had become hardened and firm, and many of them could be seen with the naked eye. When the seed from such infected bolls was examined with the aid of a binocular dissecting microscope incipient pycnidia could be seen on the testa in the region of the hilum. These pycnidia developed rapidly (Fig. 9, Plate 3) when the seed bearing them was incubated in a moist chamber, and at the end of twenty-four hours many of them were exuding pycnospores (Fig. 10, Plate 3) which on examination proved to belong to *S. linorum*. It is noteworthy that a similar type of seed-infection was found to occur in the case of *L. angustifolium*, and many seeds bearing pycnidia of *S. linorum* were obtained from diseased plants of this species.

The path of the fungus, therefore, appears to be from the sepals to the pedicel, thence to the placenta, and so to the seed through the funicle. Further confirmation of the conclusion that flax seed becomes infected with *S. linorum* in this way was

forthcoming when serial sections were made of diseased seeds. It was proved in all cases where mature seed was used that the pycnidia were confined to the seed coat in the immediate region of the hilum. A very few seeds were observed on which pycnidia developed at other points, but such seeds proved to be immature with imperfectly developed coats, and were incapable of germination. Examination of sections of infected seeds showed that the pycnidium arises as a stroma between the round cells and the fibrous layers of the seed coat, and as it enlarges the former are pushed outwards and lie along the wall of the pycnidium (Fig. 16, Plate 4). The epidermal layer which is permeated by the mycelium is finally penetrated by the enlarging pycnidium, the ostiole of which pushes through the cuticle. There is no conclusive evidence that the parasite penetrated the fibrous layers of the seed coat, and there was no trace of injury to the endosperm or cotyledons. This would account for the fact that although an infected seed might bear numerous pycnidia in its testa it nevertheless germinates in a normal manner.

Seed which was carefully removed by hand from flax bolls bearing pycnidia of *S. linorum* on sepals and pedicels was placed in a germinator at room temperature. After three days 80 per cent. of the seed had germinated, and of these 20 per cent. had developed pycnidia of *S. linorum* on their coats. Fourteen days later half of those which had developed fructifications showed water-soaked lesions on their cotyledons, and these eventually bore pycnidia of the fungus. In another experiment 100 seeds from the same source as above were sown in a pot of sterilised soil in the glasshouse and germinated to the extent of 92 per cent. The pot was covered with a belljar to exclude possible extraneous contamination and to maintain a moist atmosphere around the seedlings. The latter at first looked normal, and there was no sign of disease. Three weeks after sowing two of the seedlings showed typical pasmo lesions on the cotyledons. Three days later two other seedlings in the pot developed similar lesions, and in due course all the lesions developed pycnidia of *S. linorum*. This is conclusive evidence that infection originated in the seed.

As already shown, pycnidia of *S. linorum* can develop rapidly on infected flax seed when the latter is placed in a moist atmosphere, and it follows that pycnosporos from such fructifications would under moist conditions stream on to the cotyledons in those cases where the seed coats are carried above ground and remain attached to the seedlings. A higher percentage of infected seedlings would be expected to occur under field conditions, where growth of the seedlings in the early stages would be much slower than was the case in the glasshouse.

DISCUSSION.

Since it was first observed on the continent of Europe in 1936, the occurrence of the pasmo disease of flax has been remarkable. In the past six years it has been reported in five different countries, the most recent record being that from Ireland. The occurrence of the disease in Ireland on wild flax raises the interesting question as to how this host became infected. The field from which the infected plants were collected has been in pasture for a number of years, so that if the wild host became infected from cultivated flax growing in the same field the disease must have been present here for many years. On the other hand, infection of the wild flax may

have been initiated through the agency of wind blown spores from a diseased crop in the neighbourhood. With regard to this possible source of infection, a selection of oil flax varieties has been grown for a number of years in the vicinity of the pasture-field in question, the seed being imported mainly from the U S A. An examination of these varieties was made in 1945 in the month of June, but no trace of pasmo was found, nor has any disease resembling pasmo ever been noted on them. Nevertheless, the suspicion that the disease originated from such imported seed cannot be dismissed.

A third possibility, however, presents itself—namely that the wild flax may have become infected through linseed cake used as cattle food on this land. This is a factor which must now be considered in view of the information which has come to light as a result of the present work. It is interesting to note in this connexion also that Dr. Foister, of Edinburgh, in correspondence with one of us records the finding of the pycnidia of *S. linorum* in 1941 on flax seed which had reached Scotland for conversion into cattle food and oil.

As to the distribution of the pasmo disease in Ireland, a careful survey of fibre flax crops was made in 1945 in all the flax-growing areas of the twenty-six counties. This established the fact that the disease is by no means widespread here, as it was found in only two cases throughout the year. The first case was from a farm in Co. Cork on seedlings which were forwarded to this laboratory in the month of May. Incidentally, it should be stated that the farm where this outbreak of the disease occurred was approximately 35 miles distant in a straight line from the farm where diseased plants of *L. angustifolium* were found in 1944. Moreover, a search of the farm and neighbourhood failed to reveal any infected plants of *L. angustifolium*.

The occurrence of the disease under such circumstances suggests that the parasite was probably seed-borne. The seed from which this crop was grown was, however, produced in England, and, as the disease is not known to occur there, the origin of the disease in this case is difficult to trace. Nattrass (9) in Kenya observed a severe outbreak of pasmo under similar circumstances, and was driven to the conclusion that the fungus could only have gained entry to that country on flax seed, which he assumed had come from America.

Regarding the occurrence of the second case of a crop diseased with pasmo the specimens of affected plants only came to hand towards the end of September, 1945. The crop was an oil flax of the variety Redwing grown for feeding purposes. It was raised from seed saved in Co. Kilkenny the previous year. It is interesting to note, however, that the seed which gave rise to the 1944 Kilkenny crop emanated from the Co. Cork farm on which the pasmo-infected plants of *L. angustifolium* originally occurred. This case is a further indication of the dissemination of the parasite through the agency of infected seed, and the results of the present investigation leave little doubt as to the important part which infected seed plays in such dissemination.

The survival of *S. linorum* during the winter is possible by any of the following ways:—

- (a) As pycnospores on the surface of the seed or as infected detritus amongst improperly cleaned seed samples.

- (b) By means of diseased stubble, as has been shown by Bientzel (2)
- (c) By means of the perfect or perithecial stage of the fungus.
- (d) As pycnidia and mycelium in the walls of the infected seed.

There is as yet no experimental proof that the parasite does survive in the manner suggested in (a), and, since seed dressing would be expected to minimise the infection of a crop grown from such seed, its importance as an overwintering method is thereby lessened. As regards (b), this is not regarded as of significance in this country, since in the case of fibre flax, at any rate, the crop is pulled leaving no stubble and although crop debris may remain on the ground after pulling this is generally ploughed under in subsequent tillage operations. Furthermore, due to the rotation practised here, flax is very rarely sown for two consecutive years in the same soil. In the case of (c), perithecia of *S. linorum* have not yet been seen by us, but, since the amount of diseased material which we have examined has been relatively scanty, the possibility of the formation of perithecia on diseased tissue, particularly of flax grown for oil or feeding, cannot be ignored.

The writers are, however, of the opinion that *S. linorum* overwinters and is disseminated, in this country at least, mainly as mycelium and pycnidia in the walls of flax seed from diseased crops. Support is lent to this view by the difficulty which has been experienced elsewhere in controlling the disease by means of surface seed dressings.

Seed infection of flax originating from the placenta has been described by Lafferty (6) in the case of *Polyspora lini*, the cause of Browning and Stem Break of that crop. It differs, however, from the method of infection described here for *S. linorum* in that the fungus in the former case penetrates the wall of the seed boll and thus reaches the placenta, while in the latter the parasite reaches the placenta from the pedicel.

In view of the fact that the pasmo disease appears to have been introduced into Ireland on oil flax or on oil flax products, and also since the prolonged period of growth of this crop beyond that of fibre flax would render it more liable to severe infection, it is considered that further observations on the occurrence of pasmo on oil flax are greatly to be desired. It is hoped to carry out during the 1946 season a further survey of diseases occurring on fibre flax.

ACKNOWLEDGMENT.

The best thanks of the Authors are due to Dr. Phyllis E. M. Clinch, who prepared the serial sections of diseased seeds.

SUMMARY.

1.—The occurrence throughout the world of the pasmo disease of flax and its distribution in Ireland are discussed.

2.—The symptoms of the disease are described on seedlings and on maturing plants. The fungus infects cotyledons, stems, leaves, sepals, pedicels, and internal parts of the boll. The occurrence of a resistant stage in the growth of the flax plant is emphasised.

3.—The causal fungus is described in pure culture, and the union of germinating pycnospores is noted.

4 —Seed infection by the formation of mycelium and pycnidia in the seed coat is demonstrated for the first time. The position of the pycnidium in the layers of the seed coat is described.

5 —The manner in which *S. linorum* may overwinter is discussed, and the conclusion is reached that the pasmo disease of flax is initiated chiefly through the agency of mycelium and pycnidia in the seed coat.

EXPLANATION OF PLATES.

Plate 2.

- Fig 1.—Cotyledons of flax seedlings showing pasmo symptoms produced by spraying with a suspension of pycnospores of *S. linorum* Slightly enlarged.
- Fig 2 —Development of pycnidia of *S. linorum* on the dead cotyledon of a flax seedling which has been sprayed with a suspension of pycnospores of the fungus $\times 3$.
- Fig. 3.—Portion of flax plant at the stage of boll formation showing the blighted effect produced by infection with *S. linorum* Slightly reduced
- Fig. 4.—Enlargement of a leaf lesion from Fig 3. This shows the bleaching of the central portion of the lesion and the production of pycnidia $\times 6$.
- Fig 5.—Portion of a flax plant at the same stage of growth as Fig. 3 showing the elliptical lesions on the stem. Slightly reduced

Plate 3.

- Fig. 6.—Flax boll infected by *S. linorum* showing the development of pycnidia on the sepals and decay of the pedicel at its junction with the boll $\times 3$.
- Fig. 7.—Interior of flax boll as shown in Fig. 6. Note the elongated dried spore horns arising from pycnidia embedded in the placenta. $\times 6$
- Fig. 8.—Portion of stem of flax plant diseased with pasmo showing the spore horns arising from pycnidia in the stem. $\times 3$.
- Fig. 9.—Formation of pycnidia on the coat of a flax seed derived from a boll having the pasmo disease. $\times 12$.
- Fig. 10 —Pycnospores of *S. linorum* exuded in a tendril from one of the pycnidia shown in Fig 9 after the seed had been incubated in a moist atmosphere $\times 55$.
- Fig. 11.—Pycnospores of *S. linorum* obtained from a pycnidium on the seed coat. $\times 350$.

Plate 4.

- Fig. 12 —Germinated pycnospores of *S. linorum* showing anastomosis of the germ tubes. $\times 200$.
- Fig. 13.—Chlamydospores which have arisen from pycnospores of *S. linorum*. Note the increase in width of the former over the latter. $\times 300$.

- Fig 14.—Single spore colony of *S. linorum* on potato glucose agar. Note the exudations of pycnospores from the pycnidia in the medium. $\times 3$
- Fig. 15.—Section through the dissepiment of a pasmo-infected flax boll showing pycnidia and contained pycnospores. $\times 150$.
- Fig. 16.—Section through a pycnidium of *S. linorum* in the coat of a flax seed. Note the origin of the pycnidium as a stroma between the round cells and the fibrous layer. $\times 150$.
- Fig. 17.—Surface view of the dissepiment a section of which is shown in Fig. 15. The dark circles are the pycnidia embedded in the tissues. $\times 50$

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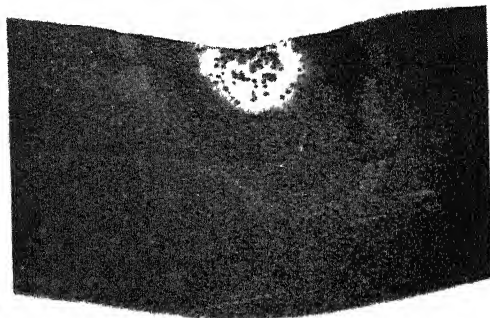
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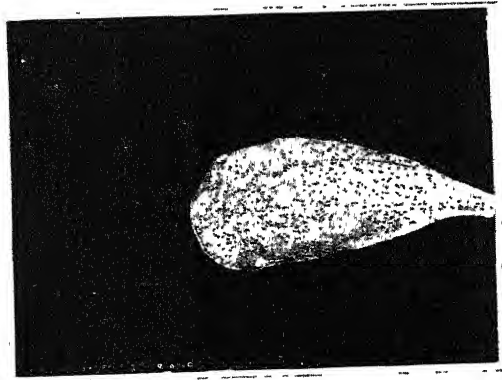
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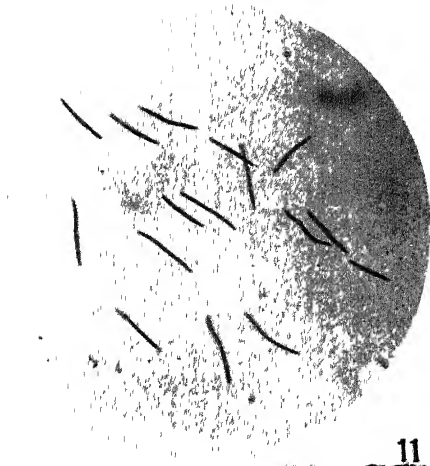
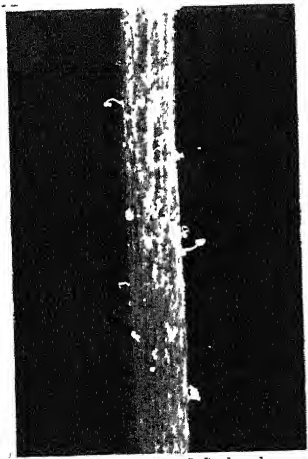
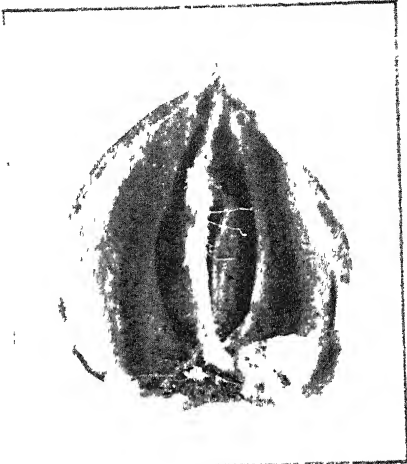
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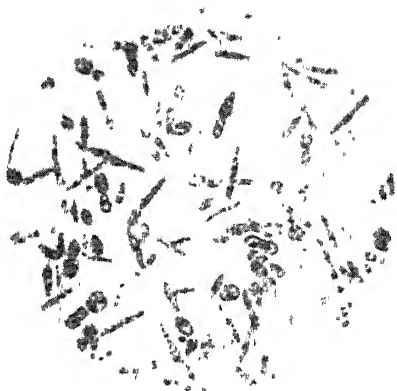


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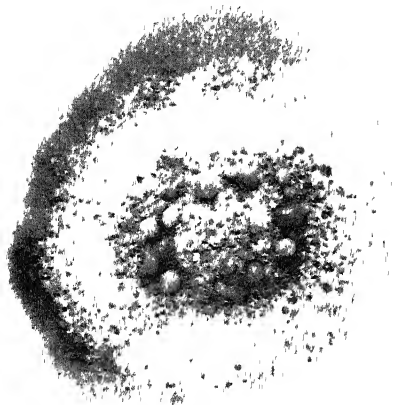




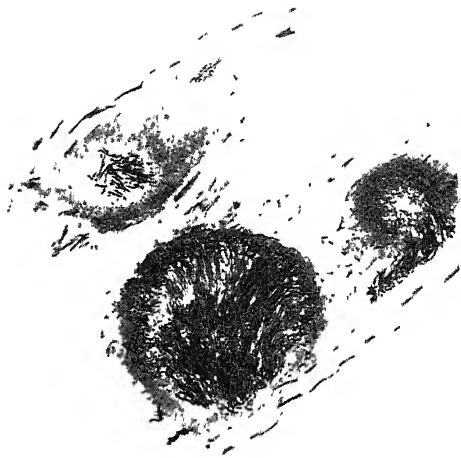
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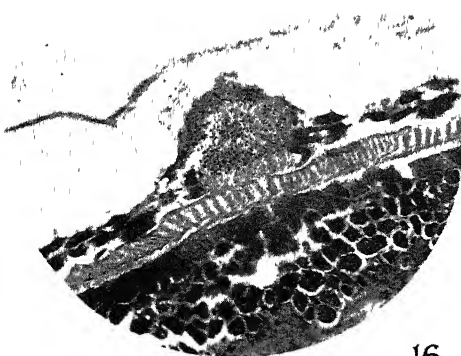
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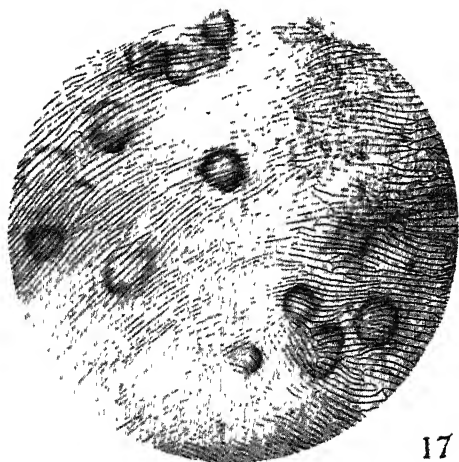
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No. II.

A STUDY OF *SEPTORIA OXYSPORA* PENZ & SACC. ISOLATED FROM
DISEASED BARLEY.

By

ROBERT MCKAY, D.Sc.

(Professor of Plant Pathology, University College, Dublin)

[Read JANUARY 29, 1940. Published separately JULY, 1940.]

(PLATES 5, 6, AND 7)

INTRODUCTION.

THE present paper deals with a disease of barley which was not observed in Ireland until the year 1931. It was recognised as a new disease in this country at that time, and as it appeared sufficiently serious to give rise to some apprehension a considerable amount of time was spent on the investigation of the fungus. The results of this work were not then published, the occurrence of the malady being simply reported as a new disease of barley associated with a species of *Septoria*, a note to this effect appearing in the Second Annual Report of the Minister for Agriculture 1932-33, Appendices page 6.

Since 1931 a careful lookout for this particular disease has been kept each year, but it was not again seen on barley until July 1945, when my colleague Mr. J. B. Loughnane collected some diseased plants in Co. Cork. An examination of these specimens showed the same characteristic *Septoria* to be present as occurred at Glasnevin 14 years previously. In this case, however, the *Septoria* was not alone, but occurred along with another fungus, *Rhynchosporium Secalis* on the affected foliage. Subsequent to some further investigation the *Septoria* was identified as a strain of *Septoria oxyspora*, and as there does not appear to be any previous account of the behaviour of this organism in pure culture, it was finally decided to publish the results originally obtained as a contribution to our general knowledge of this group.

HISTORY OF THE DISEASE AT GLASNEVIN

The disease occurred on a strain of Spratt-Archer barley. This particular selection was made at one of the Continental State Experiment Stations, and was introduced in the spring of 1931 by the Plant Breeding Division, Albert Agricultural College, Glasnevin. Incidentally the grain was treated with a dry dust preparation before sowing.

The attack was first noticed as a dark brown spotting of the foliage on the tops of a number of plants towards the middle of June 1931. When first seen only a few plants confined to an area of three square feet showed the spotting, and no particular attention was then paid to it. By the 1st July, however, the diseased plants attracted attention from a distance of twenty yards. All round the original point where spotting first appeared the top leaves of the plants were quite yellow at their tips, the discoloration varying from an inch in length on some plants to four or five inches on the leaves of others, with the result that over an area of some square yards the whole ends of the upper foliage appeared as if scorched by fire. On closer examination it was found that on the yellowish patches on the leaves innumerable small spots with a greyish centre surrounded by a purplish border occurred, and further back on the green tissue other similar spots were present. Individual spots varied in size from 1—3mm in length and were generally elongated in the long direction of the leaf. Very small spots were entirely brown or dark purple in colour. The flag leaf suffered worst (Fig. 1 Plate 5). In many cases this leaf had only a narrow band of green tissue remaining, extending from the base half-way up the lamina, all the rest being chlorotic and practically dead. Although the upper foliage was that most severely attacked, diseased spots could also be found on the lower leaves, mainly on their tips and margins. It was obvious that the killing of the foliage was due chiefly to the numerous spots which were coalescing, long narrow strips 2-3 cm. in length being killed outright. The auricles and bases of the leaves were not attacked until the remainder of the leaf was practically dead. Every plant in the plot, which covered 24 square yards, showed some of the disease on its foliage. The general appearance of the disease on these barley plants was not unlike that produced by Celery Leaf Spot on celery plants during a severe outbreak of the latter disease in September or October.

No trace of similar spotting could be found on any of the adjacent plots of barley, which comprised fifteen foreign varieties, the sources of these being three European countries, Canada, and The United States. In addition to this foreign collection there were several native varieties and hybrids. As the outbreak and general symptoms did not appear to be identical with any of the usual barley diseases, and as it was evident that the trouble was spreading rapidly, following a consultation with Mr. Caffrey, Head of the Plant Breeding Division, it was decided to destroy the entire plot. The barley was accordingly cut and destroyed. Previous to this a few plants had been potted up for investigation purposes, and the fungus had already been isolated in pure culture from typical diseased specimens.

The plot of barley which was cut on 2nd July sprouted up again from the base, and the same disease was found on the young foliage towards the end of July and again in September. The attack on this foliage always remained slight.

INVESTIGATION OF THE DISEASE.

When affected leaves are examined closely it is seen that the dead yellowish areas and the grey centre of the spots are studded with small pycnida (Fig. 2 Plate 6). These are just visible to the naked eye as small black dots, and occur in straight rows owing to the arrangement of stomata in barley leaves. Each pycnidium occupies a sub-stomatal cavity, and on the bleached greyish portion of the diseased

area almost every stoma may have one below it, so that pycnidia are often touching each other in the rows. Owing to their position a line of pycnidia is often found on each side of a leaf vein, two rows occurring between each pair of veins. The fructifications occur on both sides of the leaf, but they are more abundant on the upper surface. Pycnidia are slightly flattened on the top, and have their greatest diameter parallel to the vascular bundles (Fig. 3 Plate 6). They vary in size from $76\mu \times 72\mu$ to $119\mu \times 78\mu$, and their breadth is more constant than their length, the average width being 77μ . These measurements are from fresh material.

Examination with a compound microscope shows more or less of a pattern on the pycnidial wall, which is pseudo-parenchymatous, brownish to almost black. This wall is thin and composed of dark short-jointed hyphae interwoven together, it is usually about 6μ in thickness, and seldom exceeds 9 or 10μ . It is frequently best developed and darkest in colour around the ostiole. The latter coincides with the stomatal opening, the guard cells of which are so affected that they are rendered incapable of functioning, the aperture being never closed but fully distended, thus providing escape for the spores to the outside. When a small piece of leaf containing pycnidia is placed in water on a slide a mass of spores exudes through the stomatal opening, and in material fresh from the field the spores may be found either projecting from the stomata or a whole mass of them can be found outside the aperture.

The pycnospores are thin-walled, hyaline, typically more or less crescent shaped, (Figs 4 & 5, Plate 6), they are slightly blunt at their heel end and bluntly pointed at their apex. While the majority of the spores conform to this type, some are shaped like boomerangs, and occasional ones like hockey sticks. Spores vary in size from $15-22\mu$ long by $2-4\mu$ wide, with an average length of 18μ and breadth of 3μ . The majority of the spores are unicellular at the time of emergence, but some are to be found with one septum (Fig. 5, Plate 6), or occasionally two cross walls. Spores left in water overnight swell up, and some of them develop cross walls, the number of septa being 1-3. Germination can take place at any part of the spore, and may occur at two or more points at the same time; many spores germinate without developing cross walls. Although some germination takes place in distilled water it is much more abundant in weak sugar solutions or in barley extract agar in 24 hours. Spore germination is somewhat variable, the most usual method is for a small protuberance to appear on the spore wall, the outgrowth developing into a hyphal thread with numerous septa. This hypha may give rise to other similar hyphae, or frequently in nutrient solutions, and especially in barley extract agar, it commences to bud off conidia exactly similar to the pycnospores. Such conidia may be borne directly on the side of the hypha, or a small projection may arise on the hyphal thread, from which conidia are abstricted, the older conidia being pushed aside by the younger (Fig. 11, Plate 7). These side growths may be very numerous; they differ in size from a mere swelling on the side of the cell to one, two or more cells, exactly like those of the parent hypha, and at the end of these subsidiary growths conidia are produced. After spore germination the first-formed hypha may develop thick walls, and from this thick-walled closely septate hypha a thin branch arises, conidia becoming abstricted from its free end (Fig. 13, Plate 7). Very often such thin-walled branches are about 22μ long by 2μ wide, consist of a single cell, and resemble a conidium which has remained attached to the parent hypha.

At the end of 48 hours after germination in barley extract agar the hyphal threads vary in thickness from 3 to 12μ , the thicker strands being formed mainly of short thick-walled cells, the length of which seldom exceeds 11μ and very often is only about 6μ . Pycnospores may also germinate by a process of budding, producing one or more similar spores, (Fig 10, Plate 7) These methods of conidial production are typical of growth on barley extract agar, and at the end of 48 to 72 hours after germination of the pycnospores all trace of mycelium is hidden under the mass of conidia produced. The only difference between conidia and pycnospores is that the former are not so curved as the latter, but even in the case of pycnospores the difference in curvature is considerable. At the time conidia are abstracted, they are from 18 to 22μ long, but in nutrient solutions they quickly swell up and may reach a size of 30μ by 4μ , though about 24μ is the average length. The conidia behave similarly to pycnospores, a few develop cross walls before germination, but the majority germinate without the formation of septa, and others bud off similar conidia direct. Development of conidia may be easily followed under the microscope by mounting a small portion of thick-walled closely septate hypha in barley extract in a hanging drop (Figs. 13—17, Plate 7).

Single pycnospore isolations of the fungus were made from diseased field material on potato glucose agar, and as all separate isolations appeared identical in pure culture one monospore isolation was retained, and all the others discarded. From this single spore isolation sub-cultures were made to different media and the behaviour of the fungus studied. Other cultures derived from the same source were used for inoculating barley plants, and in every case the same diseased condition of the host resulted and the parasite was recovered without change.

Production of the Disease in Barley Plants.

The methods of inoculation followed were to raise barley seedlings in pots, and when the plants were over two inches high to inoculate, either by spraying with conidial suspensions in water or by placing a little of the medium containing the fungus direct on the leaves, the latter being unwounded. Controls were sprayed with tap water at the same time. After inoculation the pots with their appropriate controls were covered with bell-jars for 48 hours. Both methods of inoculation were successful, and spraying with conidial suspensions in water was the only one used in later work. In the first inoculation performed in September 1931 the pots were kept out of doors, and the disease only developed to a very slight extent. These plants remained outside throughout the winter with only an occasional diseased spot visible on the leaves until near the end of January 1932. By this time the plants were twenty inches high, and the disease then assumed a much more severe form on the upper leaves. In subsequent inoculations during the winter of 1931-32, the plants after spraying with spore suspensions were covered for eight days and were kept in an unheated greenhouse. In these latter cases every plant developed the disease, and it was more typical of what appeared in the field. It should be stated here, that in every case where young plants became diseased after inoculation they afterwards grew out of the disease to a great extent, and then when they were coming into ear the disease became virulent on the upper foliage. This is the period when the plant is most susceptible to the disease, and the attack was always worst

on the upper leaves and at their tips and margins. Five separate inoculations on barley plants were carried out in the unheated greenhouse during the winter of 1931-32, and the disease was produced on each occasion, the fungus being recovered without change

Following infection in the greenhouse with conidial suspensions the first signs of disease appeared on the 12th day, when numerous pale yellow spots were seen towards the tips and margins of the leaves. The middle of these spots appeared slightly depressed, and five or six days after they became visible a brown margin developed around each of them, the centre of the spot soon became grey and bleached in appearance, and pycnidia developed. The pallid spots on the foliage may be easily detected in an early stage by holding up the leaf between the observer and the light. They vary in size from less than 1mm. to 3mm. in diameter; usually when the diameter of the spot reaches a length of 1mm. the brown margin appears. Where individual diseased spots are isolated the line of demarcation between diseased and healthy tissue is fairly distinct, but where the spots are closer together this line is not so definite, the dark brown border fades into yellowish-green, the spots rapidly coalesce, and soon the whole area becomes grey and bleached, and pycnidia develop. Microscopical examination of the pale spots on the 12th day following infection showed a septate mycelium 3—5 μ wide in the leaf tissues. The fungus here is fairly uniform in thickness, mainly intercellular at first, but very soon the cells are invaded and the parasite is both inter- and intra-cellular. Hyphae invariably make their way to the sub-stomatal cavities, where numerous septa appear in them, and they become interwoven together forming a small web of mycelium at the bottom of the air space, and all stages of pycnidial development could be found. The pycnidia are quite grey at first and invisible to the naked eye, even with the aid of a good pocket lens, but they can be detected with the compound microscope, and even in this early stage of development the whole cavity of the air chamber is often filled with spores. The ostiole is directly under the stoma, and the hyphae around it become brown as do also the basal layers of the pycnidium. Finally the whole structure darkens and can then be seen with the naked eye as a small black speck. Formation of pycnidia may be detected as early as ten or twelve days after inoculation, and perfect fructifications may appear as soon as sixteen days, but it is usually about three weeks before they become visible.

Two inoculations were carried out in the open field during the month of May 1932. In one case a number of plants in a plot of barley were sprayed with a conidial suspension and covered with bell-jars for five days. Typical infection occurred and pycnidia were found sporulating twelve days later. In the second experiment a pot containing diseased plants was placed in the middle of an isolated plot of barley and the disease allowed to spread naturally. The disease was found on neighbouring plants twenty-four days after this pot was placed in position. The weather was humid at this time and favourable to the spread of the disease, which appeared worst on the tips and margins of the foliage. Three or more inches of the leaf tip became completely yellow, this soon bleached in appearance and pycnidia developed in rows. The weather then became warm and dry, and no further spread occurred until the end of July, when there was a spasmodic outbreak during showery weather. This attack, however, remained slight, and no serious development of

disease took place. Controls throughout remained healthy, and no trace of any similar disease could be found on any of the barley plots elsewhere on the farm in 1932.

Attempts were made during the spring of that year to inoculate growing plants of the following: Cocksfoot, Timothy, Oats, Rye, and Wheat, but in all cases the disease failed to go over, although barley plants were readily infected at the same time.

Cultural Characters.

The fungus grows well on a number of artificial media, but the development of mycelium is scanty on the majority of them, and in some cases almost suppressed, the organism developing by conidia budding in more or less of a yeast-like manner.

Cultures on the various media were kept and examined over a period of eighteen months, but pycnidial development could not be induced on any artificial medium. Growth on slants in all cases was better than on poured plates, on the latter the colonies enlarge very slowly and soon cease growth unless there is a very deep layer of the medium. The following is a description of the growth of the fungus on slants incubated at 20°C:—

Potato Agar—Growth slight, bacteria-like on surface of slants, colonies less than 1 cm. in diameter at end of two weeks. Mycelium chiefly on surface or slightly submerged. Hyphae much gnarled and twisted, slightly dark in colour, varying in thickness from 2 to 6 μ . Thicker branches covered with many short side growths which all produce conidia. Surface of the slants dry in appearance at end of fifty days, no aerial growth and no development of colour in medium. A number of very small sclerotia present but these are never numerous even on cultures twelve months old.

Oat Extract Agar—There is very good development of mycelium which is entirely submerged, surface of slants dull grey, flat. Hyphae of a fairly uniform thickness 3-6 μ broad. A few swollen cells of mycelial threads seen but very scarce, conidia produced in moderate numbers. There is no darkening of hyphae, as occurs on other media. On cultures six months old the mycelium was all through the medium although the appearance of tubes did not suggest this. A few sclerotia formed on surface of slant but they were scanty.

Dox's Medium.—Growth extremely poor, consisting of abnormal submerged mycelium, hyphae much gnarled, twisted and swollen, forming a growth like a flattened lichen. No conidia or sclerotia produced.

Barley Extract Agar.—At the end of fourteen days colonies over 2 cm. in diameter, moist to slimy on the surface like bacterial growths, colour dirty pink in middle, growth consists chiefly of budding conidia. Colonies reach a diameter of 5 to 5½ cm. at the end of one month, but general appearance does not change. This is the ideal medium for conidial production, and though a few minute sclerotia develop on old cultures their number is relatively small. Cultures over nine months old continue to produce conidia. A dirty pink colour appears early in the middle of the growth, but the colour never spreads to any extent nor becomes intense. In cultures under one month old hyphae which are present are hyaline and have their cell walls regular, only very rare cells being swollen. In older cultures the hyphal threads vary in thickness from 2-6 μ . The same hypha may be

quite thin, then part of it may become twice as thick with correspondingly thick cell walls, and at the end of a number of swollen cells the hypha may return to its thin-walled condition again. Hyphal threads also occur which have alternately thick and thin cells.

Ground Quaker Oat Agar.—Growth on this medium is perhaps the best of any consisting chiefly of conidia at first, but plenty of submerged mycelium is produced, which grows just below the surface of the slant. Colonies reach a diameter of 2 cm. in seven days, and at the end of three weeks cover most of the slant. Middle of growth slimy, flat, dull grey to whitish at margins. Sclerotia form rapidly on surface of slants, the majority of these have a diameter under $\frac{1}{2}$ mm. but some over 1 mm. The mycelium is uniform in thickness with a width of 4.5μ , and in the early stages of its growth very few or no short swollen cells occur. A tough "leathery" crust is formed on the surface of slants, and as the culture ages the mycelium darkens at the surface, and the crust becomes raised up into folds with innumerable sclerotia dotted over it. The bottoms of these folds often remain moist and slimy in appearance, and conidia continue to be produced on such spots for a long time. On cultures over nine months old a few dirty white pustules of the fungus may be present on the surface, the amount of aerial mycelium is always very small and only the surface of the medium blackens. Sclerotia may develop on one section of plates while other sections are devoid of these bodies; cultures raised from such sectors behave like original cultures on slants.

Potato Glucose Agar (glucose 2%).—Growth on this medium is very similar to that on ground Quaker Oat agar. In fourteen days colonies reach a diameter of 2 cm. with a trace of sclerotial formation on their surface. Growth slimy, pink to deep salmon pink in colour, with numerous conidia and sclerotia at the end of 3 weeks (Fig. 6, Plate 6). Mycelium practically all submerged with a uniform thickness of 4.5μ . The mycelium soon darkens and a tough crust forms on the surface of the slants which rises up in ridges. Growth covers most of the slant at the end of one month and conidial and sclerotial formation go on side by side, the former generally in the hollows which are moist and slimy looking. In some tubes a few whitish pustules of aerial mycelium appear, but their growth is always scanty. In cultures over nine months old the whole surface of the slant appears black owing to the innumerable sclerotia present, and also to the darkening of the submerged mycelium.

Potato Glucose Agar (glucose 4%) —At this concentration of glucose, growth is very similar to above but there is a more intense development of colour, and the conidia produced are often larger than those formed on the 2 per cent. glucose or any other medium. The difference in size of conidia is most marked in tubes held at $4.5^{\circ}\text{C}.$, (Fig. 12, Plate 7). At this temperature all sizes of conidia may be found from 15 up to 41μ long by 4.5μ wide: cultures on the same medium at $20^{\circ}\text{C}.$ show conidia only slightly larger than normal. At the lower temperature on 4 per cent. glucose agar, the spores apparently go on absorbing nutriment and increasing in size without germination and generally without the development of cross walls.

Conidia produced on barley extract agar, ground Quaker Oat agar, and potato glucose agar 2% are fairly regular in size, being 22.24μ long by 3.54μ wide. These are measurements of spores found in cultures; as mentioned previously, when first

formed the spores are usually about 18μ long, but they rapidly enlarge in nutrient solutions.

Sterilized Barley Leaves —Leaves of barley plants sterilized in tubes and inoculated with the fungus from pure cultures developed a white fluffy scattered aerial growth of mycelium over their surface. Interspersed amongst this mycelium sclerotia were found, all on the surface of the leaf. In a few cases small groups of hyphae produced conidia similar to those in pure culture, and this continued up to the end of sixty-eight days. The leaf tissues were permeated with mycelium of a thickness from 2.5 to 5μ having an average width of 3μ . Only sclerotia developed on the first batch of leaves inoculated, but some pycnidial formation was induced in subsequent lots by placing a small piece of cotton wool saturated with water in the bottom of the tubes previous to sterilizing. The pycnidia formed in rows in the leaf tissue, as in the growing plant, and produced pycnospores. Immature fructifications were present at the end of twenty-one days after inoculation, but it was not until sixty-eight days had elapsed that pycnospores were found, and only a few perfect pycnidia were then observed, the others being immature or seemingly arrested in their development. Many of the tubes of inoculated sterilized leaves contained no conidia, mycelium and sclerotia only being present.

The Sclerotia.—The shape of these as in all similar bodies varies somewhat, the majority are round to oval in section, others occur which are thin flattened structures two or three times as long as they are broad. On all media, even when produced in large numbers, the individual sclerotium remains small, usually having its greatest diameter about 300μ , and seldom exceeding 500μ in those cases where it is oblong or flattened. Sections 10μ in thickness (Fig. 7, Plate 6) show them to be true sclerotia with a distinct outer rind, sometimes unequally developed, composed of thick-walled dark brown hyphae surrounding a closely packed mass of mycelium which is thin-walled and hyaline. The outer layer may vary from 12 to 60μ in thickness. In cultures larger black bodies of 1 mm or more in size occur. These when sectioned are seen to be made up of two or more (up to 8) single sclerotia which during development have grown together. In these aggregates the adjacent corners of the sclerotia are frequently very much thickened.

Cultures from single Conidia and from Sclerotia.—During the investigation of the disease cultures derived from single conidia, produced on barley extract agar, and also from single sclerotia (the latter from cultures over 12 months old) were raised and studied alongside those derived from the original single pycnospore isolation, but no difference could be detected between them, and they all behaved alike when grown under similar conditions.

Temperature Relations of the Fungus.—Between 12° and 20°C . growth was fairly even with the optimum around 20°C . The maximum for growth was 31°C ., but above 27° growth was abnormal, the cultures becoming very pink or coffee-coloured, and the hyphae much gnarled and twisted with scarcely any spread on the slants, although conidia were produced at 31°C . The minimum for growth was not determined, but growth can take place at 4°C ., as fresh transfers of pure cultures placed at this temperature grew and increased slowly, eventually covering the whole surface of the slants. The thermal death point of the fungus is between 45° and 48°C . The majority of young vigorous cultures under two weeks

old and also of older cultures up to an age of 289 days do not survive immersion for ten minutes in a water bath at 45-46°C, but on two occasions one each of a pair of cultures heated at 47°C. for ten minutes grew. In one case the treated culture was sixty-four days old, and growth did not commence until ten days after the transfer was made, while in the second instance growth of the transfer was just visible one month after treatment. In both these cases of delayed development the mycelium which eventually grew was sterile, but on transferring again to fresh tubes of potato glucose agar or of barley extract agar the characteristic conidia of the fungus appeared.

Longevity of Pycnospores.—Diseased barley leaves collected early in July 1931 were kept in an envelope in a locker in the laboratory, and pycnospores obtained from these leaves were tested for germination periodically. Small pieces of foliage containing pycnidia were placed in water and in weak solutions of sugar and teased out with needles to enable the spores to escape, the prepared slides being incubated at 20°C. Germination was good up to the end of four months, medium at seven months, and dropped rapidly after this. At the end of nine months many of the spores swelled up in the liquids but comparatively few germinated, at the termination of eleven months the germination was only 3 per cent., and this dropped to a very odd spore after fourteen months. Germination of an occasional spore was still obtained at the end of sixteen months, but scarcely any of the remainder appeared swollen.

DISCUSSION.

Several members of the genus *Septoria* are known to attack wheat, oats, rye, barley and various grasses. Amongst them *Septoria Passerinii* Sacc.—which has large septate spores—is the one most commonly reported as occurring on barley throughout the world. Another species, however, has been recorded on this host, viz. *Septoria culmifidia* Körst (3). When the disease first occurred at Glasnevin in 1931 and the fungus had been isolated and studied in pure culture, its development on different media did not agree with that of any known cultured *Septoria*, and unfortunately cultures of *S. culmifida* for comparison were not obtainable at any of the following institutions:—Imperial Mycological Institute; Lister Institute (National Collection of Type Cultures), or Centraalbureau voor Schimmelcultures Baarn, Holland.

On the recurrence of the disease in 1945 specimens of affected foliage were sent to Kew, and R. W. G. Dennis, who replied to the enquiry, suggested that the fungus was most likely a race of *Septoria oxyspora* Penz. & Sacc., and further suggested that cross inoculations to grasses might be carried out. This had already been done with entirely negative results. Dennis also kindly forwarded a specimen of *S. oxyspora* on a leaf of *Phleum pratense*. From a comparison of this material with diseased barley foliage no morphological difference could be detected between the pycnidia or pycnospores. It is therefore concluded that the fungus responsible for the disease on barley in Ireland is a strain of *Septoria oxyspora* Penz. & Sacc.

So far as the author is aware, there is no previous record of the occurrence of *S. oxyspora* on barley in the British Isles, although the fungus is fairly common on grasses (2). It has, however, been recorded on barley in Norway (3) under the name *Septoria culmifida*. Jorstad (4) reports that in the north of Norway this *Septoria* often occurs in profusion on barley together with *Rhynchosporium Secalis*, the Maskin variety being particularly susceptible. Grove (1) was apparently the first to record *S. oxyspora* from the British Isles, the fungus occurring on *Arundo Donax*. Later, Miss Sampson (5) reported it under the name *S. culmifida* on the following grasses at Aberystwyth, *Alopecurus pratensis*, *Poa trivialis*, *Dactylis glomerata*, *Phleum pratense* and *Arrhenatherum elatius*. In a more recent publication by Miss Sampson and J. H. Western (6) the fungus on these grasses is referred to as *S. oxyspora*, the former name *S. culmifida* evidently being regarded as synonymous. Grove (2) in his description of the fungus states that frequently the spores are shaped like a boomerang, while Miss Sampson found them to be typically crescent shaped (6). In the barley fungus examined by the author both types occur, but the crescentic shape predominates.

The formation of conidia in pure culture seems to be common to a number of *Septorias*. Weber (7 & 8) who made a critical study of *Septoria* diseases of cereals and grasses found that conidial production occurred freely in the majority of the eight species studied, but all of them eventually produced pycnidia, and some of them perithecia also. Weber makes no mention of sclerotial formation in pure culture in any of the species investigated by him. *Septoria oxyspora* in pure culture formed conidia freely, often by a process of budding, but neither pycnidia nor perithecia developed apart from the host. Nevertheless, sclerotial formation was abundant on several media in addition to conidial production.

Although the first outbreak of this disease on barley was rather alarming, so much so that it was considered necessary to destroy the affected plot, the disease is not now regarded as of serious importance. It is probable that the particular strain of barley imported from Denmark in 1931 was very susceptible to the fungus, and this would account for its virulent development. From the evidence available it would appear as if the strain of *Septoria oxyspora* Penz. & Sacc. occurring on barley is more or less specialized on this host.

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SUMMARY.

A disease of barley which first appeared on plants grown from imported grain in 1931 was again found on barley plants in 1945.

The causal fungus was isolated, studied in pure culture, inoculated to barley plants and the disease reproduced. It is believed to be a strain of *Septoria oxyspora* Penz. & Sacc.

Conidia are freely produced in pure culture on several media: This development is often accompanied by the formation of sclerotia, and in this respect the fungus appears to differ from any known *Septoria* which has been cultured from the small cereals or grasses.

The disease on barley is of no economic importance at the present time in this country.

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EXPLANATION OF PLATES.

Plate 5

- Fig. 1. Severe infection of flag leaf of barley by *Septoria oxyspora* typical appearance of the disease as it occurred in 1931.

Plate 6.

Figs. 2, 3, 4, 5 and 7 photomicrographs

- Fig. 2. Diseased portion of barley leaf showing linear arrangement of pycnidia of *Septoria oxyspora* below stoma. Section cleared in Berlese's fluid. $\times 35$.
- Fig. 3. Longitudinal section through two pycnidia showing variation in size and general structure. $\times 275$.
- Fig. 4. Group of pycnosporos. Stained with Methyl Blau. $\times 190$.
- Fig. 5. A single pycnospore showing a cross wall. $\times 360$.
- Fig. 6. Culture of *Septoria oxyspora* three weeks old on potato glucose agar showing numerous sclerotia
- Fig. 7. Section of sclerotium from pure culture, 10μ thick. $\times 110$.

Plate 7.

All Figs. on this plate camera lucida drawings and all to magnification of 840

- Fig. 8. Germination of pycnosporos in 5 per cent. cane sugar solution at end of 24 hours.
- Fig. 9. Appearance of germinated pycnosporos in 5 per cent. cane sugar solution at end of 96 hours.
- Fig. 10. A pycnospore budding off conidia direct in barley extract agar.

- Fig. 11. Thick walled, closely septate hypha producing conidia in barley extract agar.
- Fig. 12. Conidia in 4 per cent potato glucose agar at end of 14 days held at temperature of 4°-5°C.
- Figs. 13-17. Successive stages in development of conidium in hanging drop of barley extract agar
- Fig. 13. Appearance at 2 0 p.m. attached spore 15.5 μ long.
- Fig. 14. 3 50 p.m. Appearance of new spore.
- Fig. 15. 4.20 p.m. New spore 11 μ in length.
- Fig. 16. 4.30 p.m. Both spores still attached
- Fig. 17. 4.40 p.m. Older spore abstricted.



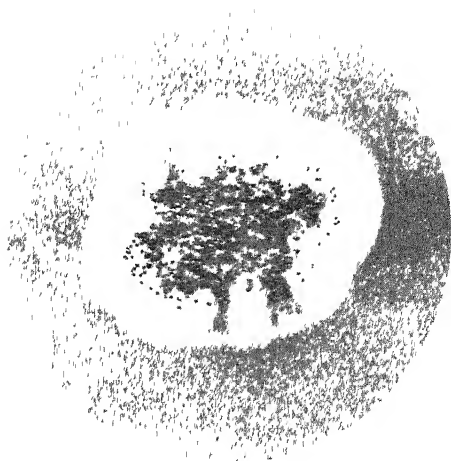
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FIG. I

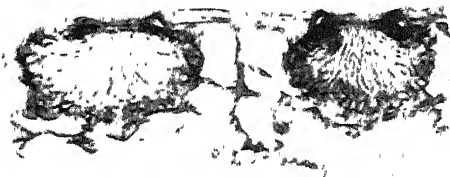
G. H. McLean



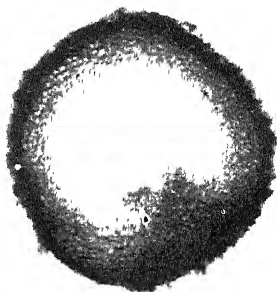
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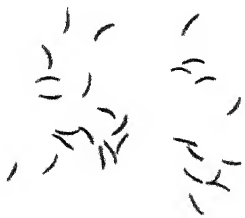
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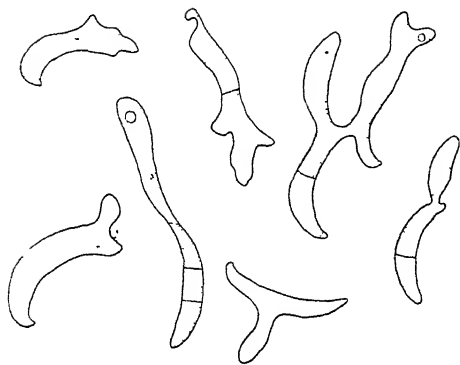
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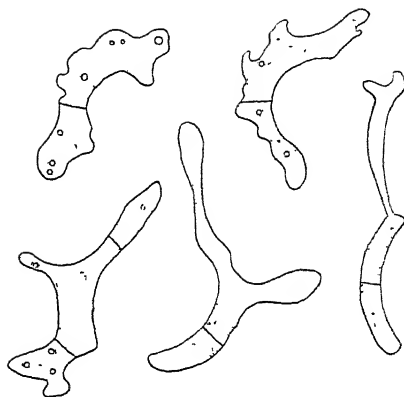
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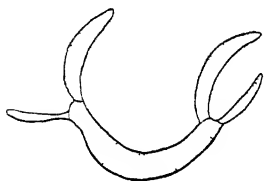
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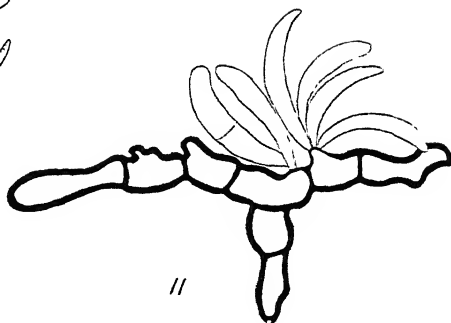
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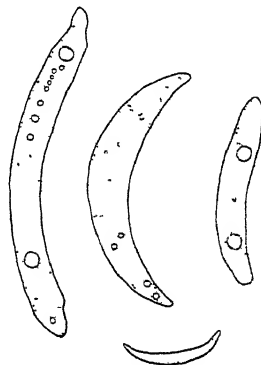
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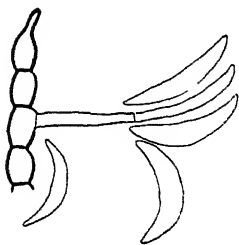
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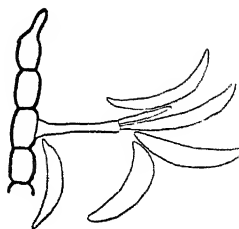
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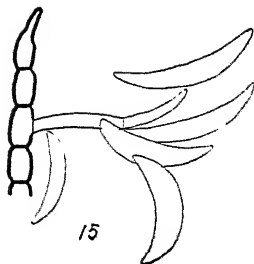
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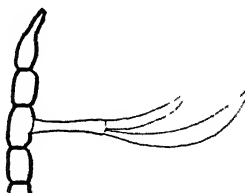
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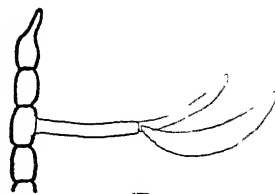
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No. 12.

STUDIES ON UREIDES.

PART 2.

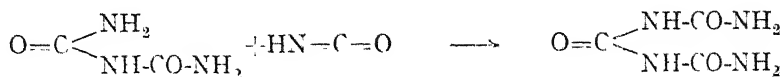
THE CHEMISTRY OF TRIURET AND RELATED COMPOUNDS

By

A. E. A. WERNER AND J. GRAY.

[Read MARCH 26, 1946 Published separately JULY, 1946]

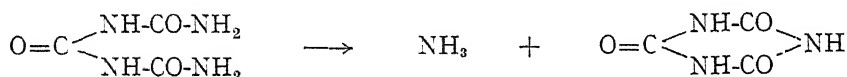
IN a previous communication (1) it was shown that when equimolecular proportions of thionyl chloride and urea were refluxed biuret was obtained. Shortly afterwards Haworth and Mann (2), using a smaller molecular proportion of thionyl chloride, confirmed the formation of biuret, but reported the formation of the compound triuret, or carbonyl diurea, $\text{CO}(\text{NH}-\text{CO}-\text{NH})_2$. Repetition of the reaction, as recorded in the present investigation, has confirmed the results of Haworth and Mann (*loc. cit.*), triuret being obtained in yields up to 30 per cent. of the theoretical. The failure to detect triuret in the original investigation may be traced to the fact that it is readily decomposed in alkaline solution into ammonia and cyanuric acid. This reaction constitutes in fact the most convenient method for the preparation of triuret; other methods described by Schmidt (3), Schiff (4), and Schittenhelm and Warnat (5) are either troublesome or give smaller yields. Haworth and Mann (*loc. cit.*) regarded the formation of biuret and triuret as involving primarily the deamination of urea; but, in view of the experimental evidence obtained by the writers (6) concerning the mechanism of the formation of allophanates by the action of thionyl chloride on carbamates, it is probable that an analogous mechanism is responsible for the formation of biuret from urea and cyanic acid, and the triuret is then formed by the addition of cyanic acid to biuret according to the following equation:—



Such a mechanism would be in agreement with the observation of Bougault and Leboucq (7) that triuret is formed along with biuret when allophanic acid chloride is treated with ammonia.

Although, as reported by Haworth and Mann (*loc. cit.*), thiourea is not attacked by boiling thionyl chloride, we have found that it is readily decomposed by this reagent at 140°-145°C forming initially ammonia and thiocyanic acid together with cyanamide and hydrogen sulphide, further secondary reactions led to the formation of a mixture of ill-defined by-products

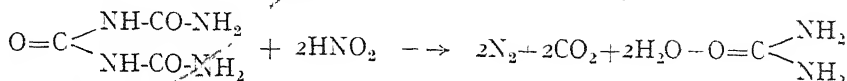
Owing to the present rather inadequate and contradictory state of the chemistry of triuret, the work detailed below was undertaken to gain more precise data about its chemical behaviour. The melting point of triuret has been recorded as 231-232°C by Schiff (*loc. cit.*), as 227°C (with effervescence) by Haworth and Mann (*loc. cit.*), while Walters and Wise (8) maintain that triuret has no true melting point, the figure recorded as such being the temperature at which triuret was supposed to undergo deamination, cyclising to form cyanuric acid with the evolution of ammonia, according to the following equation —



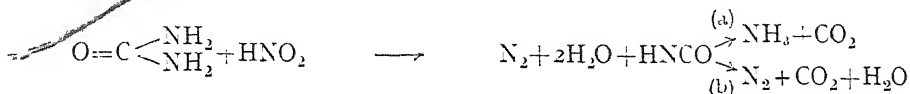
Our specimen of triuret darkened and underwent sudden decomposition at 230°C, but showed no appearance of fusion even when the temperature was raised to 250°C. This behaviour confirms the finding of Walters and Wise (*loc. cit.*) but further investigations showed, however, that the thermal decomposition of triuret cannot be represented as a mere deamination with the immediate formation of cyanuric acid as the sole non-volatile product. The detection of such by-products as ammonium cyanate and urea present in the sublimate formed on the cool sides of the test-tube, and biuret and ammelide present in the infusible residue, preclude this explanation. Cyanuric acid is quite stable up to about 300°C, and once formed would not subsequently decompose to yield the by-products above mentioned. In point of fact the triuret molecule undergoes complete disruption to give initially ammonia and cyanic acid, from which the final products are formed in a manner similar to that involved in the thermal decomposition of urea as described by E. A. Werner (9).

Since Schiff (*loc. cit.*) mentioned that triuret gives a faint biuret reaction, we have investigated the behaviour of triuret with copper salts in alkaline solution. According to our findings a violet coloration was produced when a concentrated alkaline solution of triuret was treated with only a *few drops* of dilute copper sulphate solution, but the addition of more copper sulphate solution produced a deep-blue colour, due to the formation of a copper complex compound with the triuret. This deep-blue solution was only stable for a few days. On standing black copper oxide was deposited and long acicular crystals of cyanuric acid separated from the now colourless solution. The formation of cyanuric acid is due to the slow decomposition of triuret in alkaline solution. The action of nitrous acid on triuret was next investigated and compared with the behaviour of biuret and acetamide. Although a suspension of triuret did not react with nitrous acid, even in the presence of a large excess of mineral acid, a solution of triuret in 50 per cent. sulphuric acid was found to react readily, with the evolution of nitrogen and carbon dioxide.

A certain amount of nitrogen was however fixed in the form of an ammonium salt, which was detected in the solution when the reaction was completed. This behaviour parallels that of biuret, and supports the constitution assigned to triuret. The initial reaction may be represented by the following equation:—



The urea formed then reacts with nitrous acid in the manner described by E. A. Werner (10):—



Reaction (a), the hydrolysis of cyanic acid, is responsible for the formation of the ammonium salt.

Haworth and Mann (*loc. cit.*) have directed attention to an unexpected alteration in certain properties shown by biuret, triuret, and the next two homologues in the series, namely the substances described in the literature as 'tetrauret' and 'carbonyl dibiuret.' The following tabulation is taken from their paper:—

| | M P. °C | Solubility in cold water | Biuret reaction |
|------------------------------------|---------------|-----------------------------|-----------------------|
| $\text{NH}(\text{CONH}_2)_2$ | 190 (decomp). | Moderately soluble | Deep red colour |
| $\text{CO}(\text{NHCONH}_2)_2$ | 231 (, ,). | Slightly soluble | Very faint colour |
| $\text{NH}(\text{CONHCONH}_2)_2$ | 186 (, ,). | Readily soluble | Intense violet colour |
| $\text{CO}(\text{NHCONHCONH}_2)_2$ | 235 (, ,). | Almost insoluble | No coloration |

This unexpected alteration in the specified properties, and in particular the remarkable closeness in the melting points recorded for the two pairs of substances *biuret* and *tetrauret*, and *triuret* and *carbonyl dibiuret*, would seem to warrant a more detailed investigation in order to amplify the very meagre knowledge of the chemistry of the two substances *tetrauret* and *carbonyl dibiuret*, and thereby establish definitely whether these two latter compounds do actually exist.

The first reference to the substance *tetrauret* is contained in a paper by Thiele and Uhlfelder (11), who described its preparation by the action of ammonia on allophanic azide; they recorded its melting point as 186°C, and stated that it could be distinguished from biuret by its crystalline form, its greater solubility in water, and its violet colour with alkaline copper sulphate. This reaction has been recently reinvestigated by Lipschitz (12), who treated allophanic azide with ammonia under varied experimental conditions, but in every case he could only isolate the normal amide, biuret, which was identified by chemical analysis and by mixed melting point with an authentic specimen of biuret. In no experiment was a significantly different product formed which could be claimed as the substance *tetrauret*¹. An alternative method for the preparation of *tetrauret* was described by Davis and Blanchard (13), who warmed an aqueous solution of biuret and nitrobiuret to a temperature of about 80°C. They stated that the nitrobiuret decomposed in the aqueous solution

¹ The above reaction would seem to be unique, because carbamic azide gives urea, and *not* biuret, on treatment with ammonia, according to the work of Thiele and Uhlfelder (*loc. cit.*).

to yield nitroamide and dicyanide, moist litmus paper held over the mouth of biuret to give a substance which they only decomposed with copious evolution. A brownish residue was only based on the fact that its properties were similar to those of the substance described by Thiele and Uhlenberg (*loc. cit.*) No analytical data was given, no mixed melting point determination was made, and no attempt was made to prepare any derivatives by which the compound have been more definitely characterised. We have repeated the procedure described by Davis and Blanchard (*loc. cit.*), but have been unable to obtain any product of the reaction other than biuret, which was identified by mixed melting point determination with an authentic specimen of biuret and by analysis for nitrogen content. In no experiment was a substance obtained corresponding to tetrauret. It is thus evident that the substance described in the literature as tetrauret has not been obtained by either of the methods purporting to describe its preparation, and, until further experimental evidence is produced to the contrary, the existence of such a substance must be regarded as extremely doubtful.

The original preparation of the substance known as carbonyl dibiuret was described by Schmidt (*loc. cit.*), who obtained it in small yield by heating biuret with phosgene for twelve hours at 60°C in a sealed tube. Schmidt did not record any melting point for his product, but recorded analytical data corresponding to the required formula, $C_5H_8O_5N_6$. He further mentioned that the compound was more soluble in water than triuret (which he had also prepared at the same time by reacting urea with phosgene), and that when nitrous fumes were passed for a short time into a suspension of the compound in warm water, carbon dioxide was evolved and on cooling crystals of urea nitrate were deposited together with cyanuric acid. Finally he described the formation of the insoluble compound $C_5H_8O_5N_6 \cdot 3HgO$ formed upon the addition of mercuric nitrate to a hot aqueous solution of the same substance. Schiff (*loc. cit.*) a few years later claimed to have obtained the same substance, again in small yield, by heating urea with phosgene in toluene solution for two days at 100°C in a sealed tube. He characterised the substance by recording its melting point as 235°C with decomposition, and remarking that the substance gave a feeble biuret reaction, but he did not include any analytical data concerning the compound. The meagre data presented by Schmidt and Schiff has not been verified by any recent workers, and taken by itself it is not sufficiently conclusive to put the existence of the substance described as carbonyl biuret beyond possible doubt. In view of the very probable non-existence of the so-called tetrauret it is reasonable to seek for an alternative interpretation of the observations of Schmidt and Schiff. The isolation of cyanuric acid as one of the products of the action of nitrous acid on the supposed carbonyl dibiuret is highly significant. It is very difficult to account for the formation of cyanuric acid as a normal product of such a reaction; since, for example, triuret does not yield cyanuric acid on treatment with nitrous acid although these two substances should be closely related. The only reasonable explanation would seem to be that the cyanuric acid is already present in the substance treated with the nitrous acid, i.e. the supposed carbonyl dibiuret is merely a mixture of the two compounds triuret and cyanuric acid. Such a mixture would account for the

its melting point and its apparent solubility. Cyanuric acid is appreciably more soluble in water than triuret. The analytical figures given by Schmidt, namely

$C=25.74$, $H=3.75$ and $N=37.51$, are equally in agreement with those of a mixture of cyanuric acid and triuret in the ratio 1:3, which requires $C=25.7$, $H=3.5$

$C-N=26.4\%$. Finally it should be noted that cyanuric acid and triuret are

The urea for Schmidt and Schiff for the preparation of the supposed carbonyl diuret is converted quantitatively into cyanuric acid if it is heated with phosphene, as was shown by Schmidt himself (*loc. cit.*). We are therefore led to the conclusion that the non-existence of the two substances described in the literature as tetrauret and carbonyl diuret is the true explanation of the unknown factor supposed to be responsible for the remarkable alternation in properties to which attention was directed by Haworth and Mann (*loc. cit.*) This conclusion would seem to receive a certain measure of support from the fact that no derivatives of either 'tetrauret' or 'carbonyl diuret' have been prepared, whereas well characterised derivatives of triuret have been described, e.g. 1:7 dimethyl triuret¹ has been prepared by Fischer (14) by reacting *sym*-dimethyl urea with phosphene, and a series of benzylidene derivatives has been obtained by Bellavita (15)

EXPERIMENTAL.

Interaction of urea and thionyl chloride. A mixture of dry, finely powdered urea (30 g.) and thionyl chloride (13.5 c.c.) was heated on a water-bath for 2½ hours with occasional vigorous shaking. While still warm unreacted thionyl chloride was removed by evacuation of the flask; the orange-yellow solid mass left was extracted with distilled water (150 c.c.) at 70°C, and an undissolved residue of triuret was collected (Weight=6.4 g. Decomposition point 230°C). The filtrate deposited on cooling monohydrated biuret in flat plates which in vacuum desiccator became anhydrous. (Weight=13.2 g. M.P. 185°C). On recrystallisation from ethyl alcohol the M.P. was raised to 190°C, being unchanged on mixing with an authentic specimen of biuret. The crude triuret was freed from traces of cyanuric acid by recrystallisation from boiling water, from which it was deposited in the form of small glistening needles. (Weight=5.6 g. equivalent to 23 per cent. of the theoretical yield. Found $N=38.3$ per cent. $C_3H_6O_3N_4$ requires $N=38.4$ per cent.) A repetition of the experiment gave biuret (13.8 g.=53.6 per cent. of the theoretical) and triuret (7.4 g.=30.5 per cent. of the theoretical).

Action of heat on triuret. Triuret (1.0 g.) was heated in a clean dry test-tube. Traces of ammonia gas started to be evolved at 175°C, being detected by its

¹ Biltz (16) has also prepared this substance, but prefers to represent it as the 1.5 dimethyl isomeride.

characteristic odour and the blueing of m. the test-tube. At 232°C the whole mass sudden- tion of ammonia gas, but no apparent fusion occu- mained in the test-tube, which dissolved readily in caustic soda; this solution gave strong positive tests for the presence of biuret, ammelide, and cyanuric acid. The sides of the test-tube were covered with a white deposit, which was found to contain small amounts of cyanuric acid, ammonium cyanate, and urca.

Action of heat on cyanuric acid. Cyanuric acid (0.5 g.), previously dehydrated by heating at 110°C, was maintained at a temperature of 250°C for 30 minutes. There were no obvious signs of decomposition, and tests for the presence of ammonium cyanate were negative.

Colour reaction of triuret with alkaline copper sulphate To a saturated solution (5 c.c.) of triuret in caustic soda (10%) one drop of copper sulphate solution (5%) was added. A clear violet coloration developed similar to that given by biuret. Upon the gradual addition of further amounts of copper sulphate solution the solution assumed a deep-blue colour similar to that given by ammonia and amines. Five c.c. of copper sulphate solution was added to a solution (20 c.c.) of triuret (0.23 g.) in caustic soda; excess copper hydroxide precipitate was filtered off, and the deep-blue solution was left to stand at room temperature in a stoppered cylinder for a period of two weeks, during which a black precipitate of copper oxide settled out together with long white crystals. These crystals were identified as cyanuric acid. Weight=0.237 g. equivalent to 92% of the theoretical yield, assuming that one molecule of triuret gives rise to one molecule of cyanuric acid dihydrate, $(\text{HNCO})_3 \cdot 2\text{H}_2\text{O}$.

Interaction of biuret and nitrobiuret in aqueous solution. Nitrobiuret (1.4 g.) and biuret (1.2 g.) were dissolved in warm water (50 c.c.) When effervescence had ceased the solution was evaporated down to a volume of 10 c.c. A crystalline material separated. Weight=0.98 g., M.P. 175°C (Davis and Blanchard (*loc. cit.*) reported weight =0.60 g. M.P. 178°-180°C). Recrystallisation from ethyl alcohol gave a product M.P. 188°C, not depressed by mixture with an authentic specimen of biuret. Found N=40.6 per cent. $\text{C}_2\text{H}_5\text{O}_2\text{N}_3$ requires N=40.8 per cent., whereas a compound of the formula of 'tetrauret,' namely $\text{C}_4\text{H}_7\text{O}_4\text{N}_5$, would require N=37.0 per cent.

Action of nitrous acid on triuret, biuret, and acetamide Acetamide (0.059 g.) biuret (0.05 g.) and triuret (0.073 g.) were dissolved in 50 per cent. sulphuric acid (4 c.c.), and treated in a nitrometer with an aqueous solution (2 c.c.) containing one milligram molecular proportion of sodium nitrite. After thirty minutes the volume of gas evolved was noted, and the gas analysed, the carbon dioxide content was determined by noting the decrease in volume after treatment with a saturated solution of potassium hydroxide, and the amount of nitric oxide was determined by absorption in a concentrated solution of potassium permanganate. All volumes

were reduced to N.T.P., and the actual results (averages of three experiments) are tabulated below:—

| Substance | Volume of Gases in c.c. | | |
|-----------|-------------------------|------|----------------|
| | CO ₂ | NO | N ₂ |
| Acetamide | 0 | 3.10 | 11.20 |
| Biuret | 14.50 | 1.20 | 18.45 |
| Triuret | 10.15 | 3.6 | 15.95 |

Interaction of thiourea and thionyl chloride. Dry, finely powdered thiourea (7.6 g.) was heated with thionyl chloride (13 c.c.) in a sealed tube at 140°C for 1½ hours. Vigorous reaction occurred with the formation of an orange-yellow product. On opening the tubes gas under considerable pressure was released (in one case the tube was completely disintegrated with great explosive force.) The residue was extracted with boiling water (75 c.c.) and filtered while hot. An insoluble yellow amorphous powder remained. Weight = 3 g. Insoluble in organic solvents. On heating deposit of sulphur forms on the walls of the test-tube, and a dark-brown infusible residue remains. The filtrate gives a positive test for the presence of thiocyanate and sulphide, and upon neutralisation with sodium hydroxide a white precipitate is formed, which redissolved on warming, giving a yellow solution.

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No. 13.

EVIDENCE FOR A MITOTIC HORMONE:
OBSERVATIONS ON THE MITOSES OF THE EMBRYO-SAC OF
FRITILLARIA IMPERIATIS

By

HENRY H. DIXON, ScD., F.R.S.

Professor of Botany in the University of Dublin

PLATES 8, 9, AND 10

[Read NOVEMBER 27, 1945.

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IN the year 1894 I came across peculiar modifications of form in resting and mitotic nuclei of the endosperm of *Fritillaria imperiatis*. A short account of some of them was given in a paper read before the Royal Irish Academy in November, 1895.

In the study of the preparations made for these observations remarkable examples were found in which the successive stages of mitosis followed one another in faultless sequence in the nuclei embedded in the protoplasmic lining of the embryo-sac (Pl 8, Figs. 1-9).

To investigate the problem as to how such an orderly arrangement was brought about a considerable number of preparations were made. The material consisted of young seeds from the green capsular fruits of *F. imperialis* collected when the seed measured approximately 5 x 3 x 2 m.m. These were fixed in 95% alcohol. Quick penetration and fixation of the cytoplasm and the nuclei of the embryo-sac were secured by the puncture of the seed with a needle as it was submerged in the alcohol.

Some of the seeds after fixation were split with a scalpel or razor parallel to the two flat sides of the seed, cutting through the embryo-sac down almost to the funiculus. The sides of the seed were then prized apart and under 50%-70% alcohol the membranous protoplasmic lining was dissected out from the nucellus. In this way good preparations of the lining were obtained in the form of two dished ovals cohering by their fold on the funicular side. After transference through several changes of water they were stained in dilute Delafield's Haematoxylin. When

the stain, judged by direct microscopic examination in water was judged to be sufficiently intense, the linings were washed, dehydrated, cleared in oil of cloves, and mounted in balsam.

Such preparations of material fixed in the early stages of growth of fertilized ovules afforded wonderful examples of the orderly sequence of the successive stages of mitosis distributed in the cytoplasmic lining of the embryo-sac (Pl. 8, Figs. 1-9).

The nuclei are very uniformly scattered in the protoplasm. In the earlier stages there are no cell-walls separating them. Many of them may be undergoing mitosis. The fact that the nuclei in each stage of mitosis form parallel bands in the film forces itself upon the observer. Each band is composed of 1, 2, 3 or more parallel lines of nuclei in the same stage of nuclear division, prophase, metaphase, anaphase and telophase. Generally the prophase band is composed of 4 or 5 lines of prophase nuclei, the aster band of one or two aster lines, the metaphase, anaphase and diaster bands of one or two lines each, and the telophase band of four or five lines merging finally into a wide region occupied by resting interphase nuclei. The correspondence between the succession of the stages with their spatial distribution is challenging.

Plate 8 shows microphotos of nine successive stages of mitosis from successive bands from one of these films

Like many other embryo-sacs the uninucleate condition of the embryo-sac of *F. imperialis* appears to give rise to a coenocyte with 8 nuclei. The secondary nucleus of the sac is formed by the fusion of two of these nuclei. By successive mitosis this fusion-nucleus (the secondary nucleus of the embryo-sac) gives rise to the numerous nuclei of the endosperm. These are distributed through the growing protoplasm of the enlarging embryo-sac. Meanwhile the liquid contents of the sac coalesce to form a large central vacuole and by their rapid accumulation reduce the protoplasm to a thin film, or pellicle, lining the sac.

(Plate 10, Fig. 1) shows a longitudinal section of young seed cut at right angles to its median plane which passes through the centre of the sac and the axis of the funiculus. This section shows the scattered nuclei in the more or less distorted lining, which has drawn away from the surrounding nucellus. Part of this lining is seen as a continuous sheet of cytoplasm, and parts of it are seen in section. In the latter parts the nuclei appear as enlargements on the protoplasmic film. Possibly in the living state the protoplasm, being spongy and vacuolate, completely encloses the nuclei. Fixation and dehydration processes have probably been responsible for this change. Elsewhere the nuclei are seen against a more or less continuous sheet of cytoplasm. It may be noticed that in this, as in many similar preparations of this stage, a succession of consecutive stages of mitosis from the base to the apex of the sac is apparent. The earliest stages, viz., resting and prophase nuclei, are at the base of the sac, aster, metaphase, anaphase, and diaster stages are seen in its middle, while at its apex, in the region round the embryo, disperm, telophase and completely resting nuclei only are found.

Such a distribution of mitotic stages suggests that the initiating cause of mitosis travelled from the position of the final stage represented to that of the earliest one in the sequence observed. Furthermore it may be noted this very complete graduation of all the mitotic stages is only found before the development of the later com-

partmented structure, and while all the nuclei are equally exposed to substances dissolved and diffusing in the cell-sap filling the embryo-sac

It is not till after the embryo-sac has become greatly enlarged, and many hundred nuclei have been formed by successive mitoses, that separating cell-walls are formed in the protoplasmic lining film. Plate 9, Fig. 3 shows the beginning of this change, as seen from the inside of the embryo-sac vacuole. At first only the radial walls are formed mapping out considerable regions of the film into polygonal stalls round each nucleus, but apparently leaving each nucleus for some time accessible to the dissolved substances in the vacuole of the sac. Graded mitoses in these open cells are very evident in Plate 9, Fig. 4. This state of things is also rendered very apparent in transverse sections of the sac, made at this time or a little later, Plate 10, Fig. 2. Here we see the lens-shaped cross-section of the sac, in which the antifunicular fold is lined by a single layer of cells, separated from one another by tenuous radial protoplasmic films and plates, but with no defined cell-wall shutting them off from the central vacuole. The funicular fold, on the other hand, is more or less filled with thin-walled parenchyma, but even in this parenchyma the cells adjoining the vacuole are often open to it, and in sectioning their nuclei are frequently displaced into it.

The grading of the mitotic stages in these open cells, whether they belong to the funicular parenchyma, or to the antifunicular layer, is in harmony with the view that they are accessible to a diffusing, initiating, vacuolar substance, or hormone. Plate 10, Fig. 3 showing six consecutive cells isolated from the antifunicular layer is a fortunate and striking example.

In these transverse sections of the sac, while the endosperm is still non-septate, or composed for the greater part of a single layer of cells enclosing the central vacuole and opening into it (Plate 10, Fig. 2), the location of the recording mitotic stages indicates that the starting point of the diffusing hormone, while near the apex of the sac, is often situated on the side remote from the funiculus and that the hormone seems to pass downwards towards the base of the sac and inwards towards the chalaza and funiculus. In the cases observed the point of entry was not exactly opposite to the funiculus, but a little displaced to one side of the sac (Plate 10, Fig. 2). Hence it is that in these transverse sections of the sac the telophases are spread more on one side of the sac than on the other. Consequently we must imagine the front of the diffusing hormone to be oblique to the median plane of the sac.

As long as the lining is not subdivided into cells the axes of the mitoses are parallel to the outer wall of the embryo-sac (Plate 9, Figs. 1 and 2) and, as nuclear division proceeds, cell-walls begin to be formed at right angles to and crossing the mitotic spindles (Plate 9, Figs. 3 and 4). The resulting nuclei thus come to lie between these recently formed radial walls. These radial walls make connection with the outer wall of the sac, and each nucleus thus finds itself in a polygonal cell closed on one side by the continuous cell-wall on the outside of the embryo-sac, but on the inner side open to the vacuole of the sac (Plate 10, Fig. 2). If the cell-wall of the embryo-sac is growing rapidly the following mitoses like their predecessors have their axes parallel to the wall of the sac (Plate 9, Figs. 2 and 4) exemplify this result. If, on the other hand, expansion of the sac in a tangential

direction is forbidden, increase in a radial direction is favoured, and accordingly the axes of new mitoses are oriented radially (Plate 10, Figs 2 and 3). These radially oriented spindles lay down cell-walls in a tangential plane which close the cells previously opening into the vacuole and shut into the vacuole one of every pair of resulting nuclei. The process is repeated, till the tissue of the endosperm increased layer by layer, is complete.

The independent mitosis of closed cells (Plate 9, Figs 5 and 6) affords no evidence that they are initiated by a substance diffusing and spreading through the tissue of which they are part. This independence of divisions occurring in nuclei located in closed cells, coupled with the fact that nuclei, which are *not* thus cut off from the vacuole by cell-walls never fail to show co-ordination with one another, seems to me to afford strong evidence that these co-ordinated mitoses owe their initiation to contact of a dissolved diffusing substance which is arrested or much delayed by continuous cell-walls or their protoplasmic lining. Furthermore we cannot assign the co-ordination to the action of mitogenetic rays produced by one mitosis and stimulating its neighbours, for the experiments which are supposed to have demonstrated the existence of these rays show that they are effective on nuclei located within closed cells of a tissue, and no co-ordination of the mitoses of these is recorded.

From the observations recorded it is evident, that some relationship must exist between the rate of change of one mitotic stage into those which follow and the velocity of the movement of the initiating substance. If its rate of spread were high all the nuclei would receive the stimulus almost simultaneously, and the mitotic stages throughout the whole protoplasmic film would almost synchronise. On the other hand, if the movement of the hormone were *very* sluggish, a long time would elapse between its successive contacts with the nuclei in its path and the mitosis of the first recipient would be over and complete before that of the next was started. Thus if we know the time intervals between the stages of mitosis and their distances from one another we can estimate the rate of movement of the hormone or initiating substance. Several observers have recorded the times taken for the complete process of mitosis and for the intervals between its successive stages. As might be expected the rates are dependent on the temperature.

The following figures for the duration of mitosis are quoted from Introduction to Cytology by L. W. Sharp, 3rd Edition, 1936. Nuclei in the staminal hairs of *Tradescantia* 30 minutes at 45°, 75 minutes at 25° and 135 minutes at 10°. Martens (La cellule 38, 1927) found the time from the beginning of prophase to the end of telophase was 78 to 110 minutes. M. and W. Lewis (1917) recorded that complete mitosis in the mesenchyme of the chick was accomplished in 70-180 minutes. So we may take it that at air temperatures mitosis is completed in one or two hours.

In the protoplasm on the wall of the embryo-sac of *Fritillaria imperialis* and of *Helleborus orientalis* the distance from the early prophase stages of the mitoses described in this paper to the final stages of telophase measures 1.0 mm. to 1.4 mm. Hence according to the suggestion put forward the hormone must have travelled this distance in one or two hours.

It is of interest to note that the speed of diffusion of various colloidal dyes into water held in a gelatin gel varies from 0.1 mm. for Erythrosin (mol. wt 880) to 0.34

mm for Chrysoidin (mol. wt 212). While the diffusion speed of solutions of various chlorides varies from 1.0 mm to 1.4 mm per hour (Pflanzenphysiologisches Praktikum Theil II p. 3, by L. Brauner, 1932). If these figures are applicable to the hormone of mitosis, it would appear that it must be a substance in the crystalloidal and not in the colloidal state.

While one is generally impressed with the regularity of the parallel lines formed by the successive stages of mitosis in the coenocytic film of the embryo-sac, these lines sometimes become zig-zag and even greatly flexed forming sinuous curves, so that in extreme cases tongues of metaphase and even anaphase may penetrate the bands of prophase nuclei. With the ageing of the endosperm and its more complete septation these irregularities become more frequent, so that we may find groups of prophases surrounded by later stages and even sporadic mitotic nuclei isolated in a tissue, the majority of whose nuclei are resting. It seems legitimate to assign the bulk of these irregularities to the resistance opposed to the equable spreading of the hormone by the multiplying cells and membranes. Even in earlier stages the orderly march of diffusion must often be interfered with by accidental blows on the walls of the fruit and local temperature changes. The latter will not only speed up or retard diffusion but may also, influence the rate of mitotic change.

Preparation of pieces of the endosperm film containing all the nine stages figured in Plate 9 uniformly present roughly equal numbers of each. This indicates that each of the stages here figured occupies an interval of time approximately equal to the total duration of mitosis divided by nine, i.e., in a mitosis lasting 110 minutes (cf. Martens above) the dividing nucleus spends approximately 12 minutes in each of these stages.

In conclusion, I would like to acknowledge my indebtedness to Sir Frederick W. Moore, Sc.D., who on many occasions supplied me with all stages of developing fruits of *F. imperialis*. I have also to express my thanks to Mr. Joseph Murray for his assistance in producing the photographic records in this paper.

DESCRIPTION OF PLATES.

PLATE 8

Figs. 1-9. Microphotographs of nine stages of mitosis, selected from one preparation of the protoplasmic lining of the embryo-sac of *Fritillaria imperialis* x 820

PLATE 9.

Fig. 1, *Helleborus orientalis*. Nuclei and mitoses in the protoplasmic lining of the embryo-sac. No separating walls have yet appeared. Prophase mitotic stages are seen below, metaphases across the middle of the field and anaphases above. x 140.

Fig. 2, *Fritillaria imperialis*. Nuclei and mitoses in the protoplasmic lining of the embryo-sac. No separating cell-walls have yet appeared. Prophases are seen

at VI o'clock in the field, metaphases just below the diameter, anaphases above the diameter and telophases in the upper quarter of the field. x 75.

Fig. 3, *Fritillaria imperialis*. Nuclei and mitoses in the protoplasmic lining of the embryo-sac. Separating radial walls between the mitoses are seen in the upper half of the field. The same sequence of mitotic stages are found from below upwards as in Figs. 1 and 2. x 50.

Fig. 4, *Fritillaria imperialis*. Nuclei and mitoses in the protoplasmic lining of the embryo-sac. Separating cell-walls are seen between the mitoses. Sections at right angles to the lining film of protoplasm at the same time show that there are no cell-walls separating these mitoses from the central vacuole of the embryo-sac. The same sequence of prophase, metaphase, anaphase and early telophase from below upwards is observable. x 150.

Fig. 5, *Fritillaria imperialis*. Nuclei and mitoses in tissue of growing nucellus. The mitoses are sporadic among the resting nuclei. No harmonizing control is apparent. x 150.

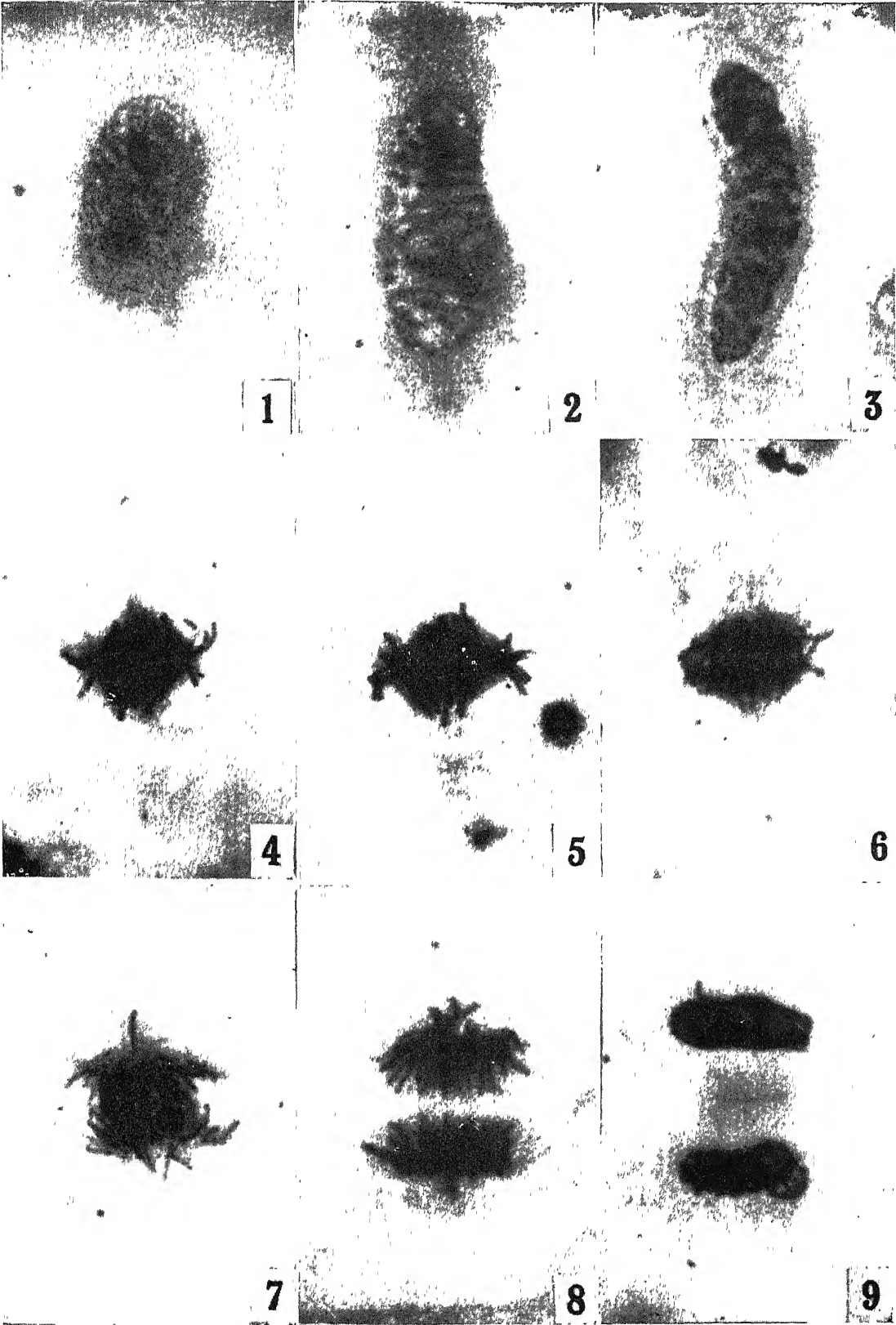
Fig. 6, *Fritillaria imperialis*. Nuclei and mitosis in tissue of growing nucellus. Single diaster is not supported by earlier stages. It may be noted that at least 30 minutes elapse between the earliest prophase and the diaster stage. Hence if a hormone evoked this mitosis it has failed in 30 minutes to traverse the walls enclosing the adjacent cells still exhibiting interphase nuclei. x 200.

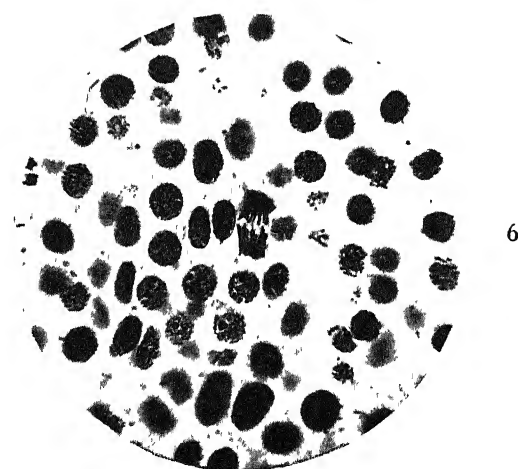
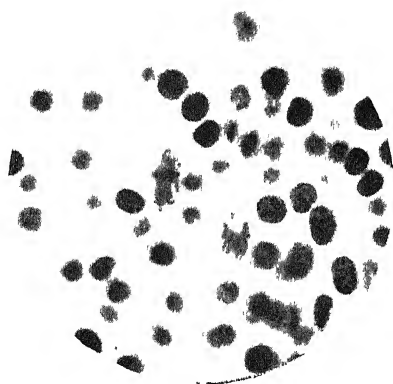
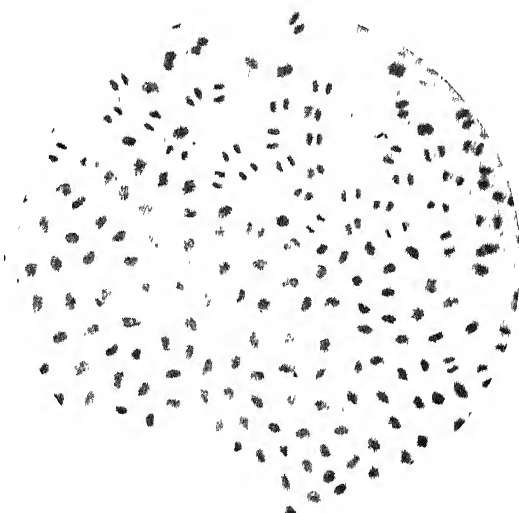
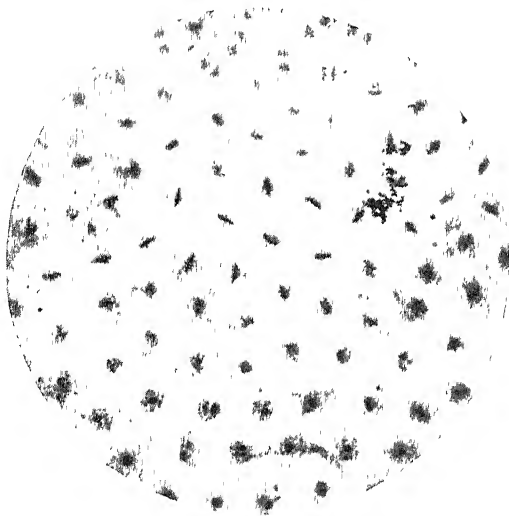
PLATE 10

Fig. 1, Microphotograph of a tangential longitudinal section of the embryo-sac of *Fritillaria imperialis*. Successive stages of mitosis are exhibited by the endosperm nuclei—the prophases at the base and subsequent stages upward throughout the sac in regular sequence. A slow movement of the hormone downwards in the sac is indicated. x 40.

Fig. 2, Transverse section—reconstructed from two adjacent microtome sections—of the embryo-sac of *Fritillaria imperialis*. The endosperm, which is massive in the funicular fold of the sac, is only one cell thick on its radial sides and in the antifunicular fold. No cell-walls cut off the cavities of this single layer of cells from the large central vacuole, and it may be seen that the successive contacts of the diffusing vacuole hormone have been able to produce the usual orderly mitotic sequences in these cells, and to harmonize the sequences of the stages in the cells of the two opposite sides. x 50.

Fig. 3, Microphotograph of six adjacent cells dissected from the wall of a transverse section of an embryo-sac of *Fritillaria imperialis*. The sequence of mitotic stages indicates the passage of the mitotic hormone from right to left. x 250.

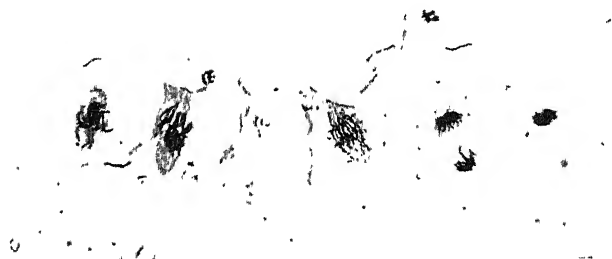






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No 14

THE OCCURRENCE OF NICKEL AND MAGNETITE IN SOME IRISH SERPENTINES, IN CONJUNCTION WITH A MAGNETIC SURVEY.

By D. W. BISHOPP, A.R.S.M.

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INTRODUCTION.

THE serpentinite of Slishwood, Co. Sligo, was the subject of a paper by E. T. Hardman with supplementary notes by Prof. Hull, published in the Proceedings of the Royal Dublin Society for 1883. In this, Hardman drew attention to a high content of magnetite (up to 15%) in the rock, and to a percentage of nickel reaching up to 2.5% in some partial analyses. Two complete analyses showed 1.00% and 1.25% nickel oxide, but the exact localities are not precisely specified.

For some years very low grade nickel-silicate ores assaying less than 2% metal have been worked in the Ural districts of Sverdlovsk, Outaley, and Orsk, U.S.S.R. A deposit of similar character has recently been described from Dillon, Montana, U.S.A.

Early in 1942 the Geological Survey carried out an investigation of the Slishwood and other serpentinite occurrences in Counties Sligo and Leitrim. Samples were taken for analysis, and the magnetic profiles across some of the occurrences were determined with a Watts vertical variometer. In 1943 a further set of samples was analysed from the belt of serpentinite rocks which occurs on the flank of the Croagh Patrick range between Westport and Louisburgh, Co. Mayo. This is the south-westerly extension of the same range of metamorphic and intrusive rocks—the Sheve Gamph or Ox mountains—which are traversed by the Slishwood serpentinite south of Lough Gill, Co. Sligo.

The object of this investigation was primarily to determine whether there were any points where concentration of metallic ores might be expected.

DESCRIPTIONS OF OCCURRENCES, AND MAGNETIC CHARACTERISTICS.

(1) *Slishwood* (1" Map No. 55, 6" Sheet Sligo No. 21, 25" Sligo XXI, 2, 6, 10.) This occurrence, which forms the subject of Hardman's paper, is the most extensive of the serpentines in this area. It is intrusive into quartzose and granitic gneisses, it has a width of 150 metres at its northern end in Lough Gill, and about

110 metres at the southern limit of the outcrop, which is 2,500 metres long. At this point, magnetic observations have indicated its extension for at least another 400 metres southward below the supervening cover of Carboniferous sediments.

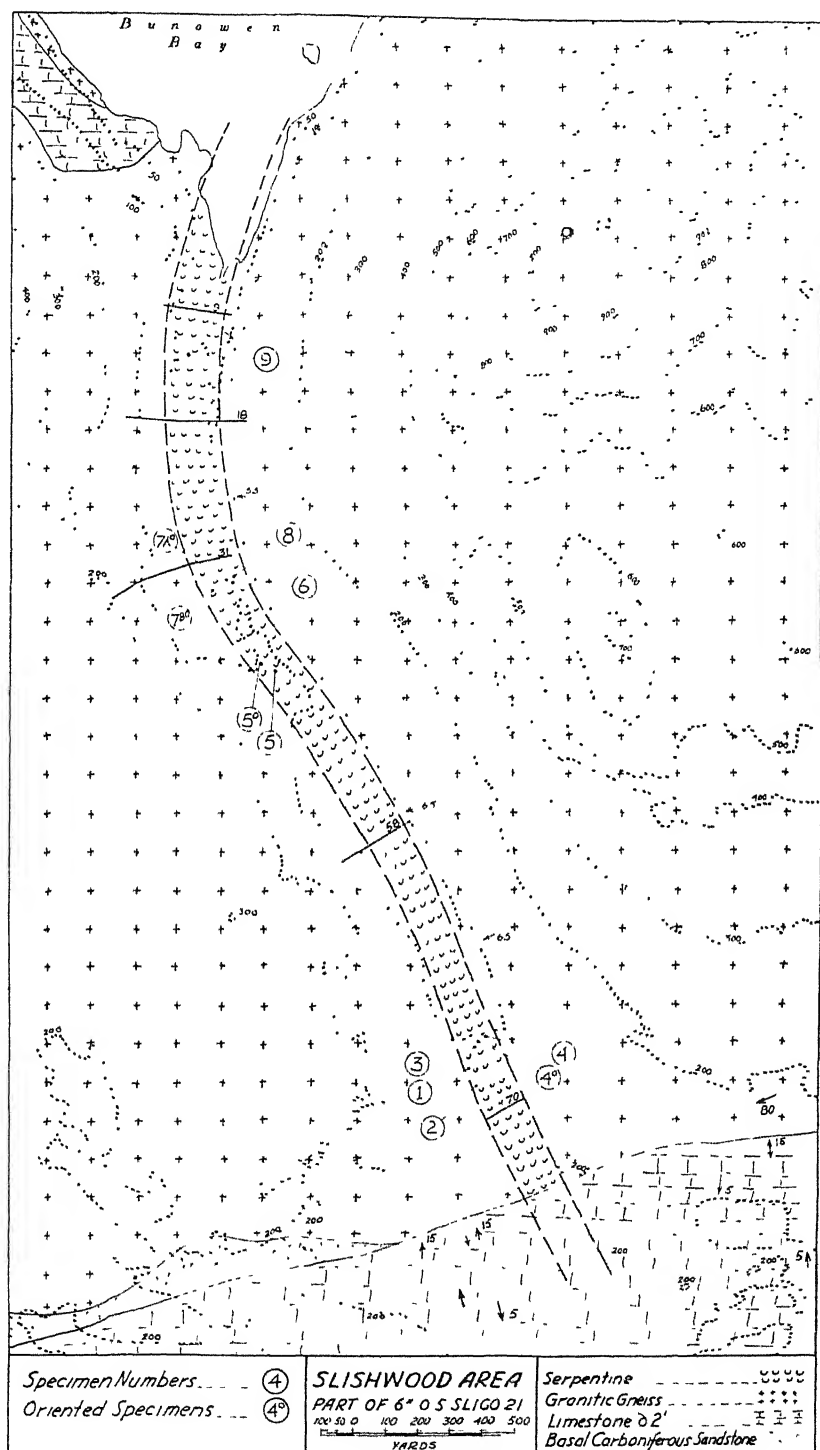
Along the centre of this belt the serpentine is dark green to black, hard, dense, and homogeneous in texture. It is nowhere exposed in contact with the gneiss, but toward the edges becomes lighter in colour, more granular, and of a banded appearance. It tends to split along planes parallel to the foliation of the gneiss, giving a pseudo-stratified appearance. At its southern end these planes dip at 70° to 90° W; at the northern end at 45° to 50° in the same direction.

In hand specimens the rock shows small crystals and fine threads of magnetite fairly evenly distributed. Thin talcose bands occur, and narrow strings of cross-fibre chrysotile asbestos are common near both margins, and are best seen near the western edge of the south end of the serpentine belt. Here, between 5 and 12 metres to the east of the contact (which is not exposed) with the gneiss, a width of 2.5 m. contains 75 bands of chrysotile from 1.3 mm. and rarely up to 5 mm. thick.

Thin sections and polished specimens of the fresh rock give rather meagre information. They show scattered opaque dust and crystals of magnetite in a comparatively clear serpentine ground mass of characteristically low birefringence. There may be a fine network of small crystals of magnetite together with crystals of opaque mineral up to 2 mm. diameter, which may also be magnetite, but tests for chromite or ilmenite have not yet been carried out. Hardman records the percentage of magnetite as varying from 2-15, adding that the higher concentration occurs in samples from the west of the serpentine belt. Nine analyses of typical examples from Slishwood in the course of the present investigation yielded an average of 8.33% total iron, equivalent to 8.0% magnetite, the values ranging between 7.4 and 10.7% magnetite.

Preliminary traverses (Nos. 70, 58, 9, 18, 31) on the accompanying map were made across the belt with observations at 10 m. intervals, and were continued over the gneiss in either direction, in spite of difficulties due to terrain. Readings over the gneiss as far as 150 m. from the serpentine boundaries were far from constant, and these variations were attributed to disseminated magnetite, which was later found there in some quantity. A normal value for the vertical component of the magnetic field was obtained over gneiss near the southern end of the belt, and over the adjoining limestone; both these formations yielded constant readings which agreed within the limits of accuracy required. The profiles drawn for the traverses across the serpentine were very irregular and of little value.

On Traverse 31 a completely different anomaly was encountered, and an attempt to measure it in detail by readings at 5 m. intervals was made, but proved impracticable. Within certain areas marked on the maps, larger anomalies were encountered which must be treated as distinct from the ordinary mass. These anomalies were in places of both signs and outside the limits of measurement of the instrument used ($-15,000 \gamma$ to $+15,000 \gamma$.) In spite of their intensity they were of very small extent, thus indicating the disturbance to lie close to the surface. Trial profiles of one anomaly were uninterpretable. In consequence the remaining traverses were made in order to outline areas where similar anomalies occurred, the grid-spacing being approximately 30 m. while readings were taken at 20 m. intervals and shortened to 10 m. as soon as abnormal readings were encountered.



Based on the Ordnance Survey by permission of the Minister for Finance.

Over the belt of alluvium which covers the serpentine near its southern end, only a few traverses were made, principally in order to check the width of the magnetic formation.

The magnetic survey showed the anomaly over most of the serpentine belt to be up to $+1,000 \gamma$, being higher near both boundaries than at the centre. This seems rather small in view of Hardman's analyses; but samples broken off and brought near the variometer produced only very small effects. The great erratic anomalies may have some relationship with the strike of the serpentine belt, since for example the areas they occupy (the sign being disregarded) are lens-shaped although very irregular, with their longitudinal axes approximately in the line of strike. These areas, as can be seen from the map, were only found near the boundaries on either side, and not down the centre, which consistently seems to be the only homogeneous part of the belt of dyke. In some of these areas the magnetic field, as traced out by a compass needle, was similar to the field produced by a North-South horizontal dipole of about 10 m. separation whose South pole pointed North. However at another area the field was similar to that of a single South pole.

The horizontal component of the field is also very disturbed and in places a compass needle is deflected 180° . The vertical field anomalies at points only a few metres apart may differ in sign and value by over 30,000 γ . The samples collected in these hyper-anomalous areas showed no very marked difference in character from those obtained elsewhere.

Several traverses were made across the serpentine areas at Aghamore, Stonepark, and Pollboy (q.v.). The anomaly here was of the same order as that generally encountered at Slishwood; but no very high values were recorded.

(2) *Aghamore*. (6" Sligo No. 21). With the exception of one small outcrop interpolated by McHenry on Hardman's map the occurrence had not previously been recorded, and the variometer observations are responsible for its extension.

Here a belt of serpentine runs along the foot of a steep scarp of gneiss. The limits of the belt are not evident through heavy drift and talus, but outcrops were observed over a distance of 400 m., and the belt appears to continue to the north-east and south-west. The contact rocks on the south-west are coarse quartzitic gneisses which dip north-west at 85° . The north-western contact is somewhat problematical. The serpentine lies very close to the fault-junction between the Carboniferous limestone and the metamorphic rocks, but whether it abuts against the fault or is separated from it by a band of gneiss has not been determined. One traverse run across the serpentine belt indicated a width of about 80 m. In appearance the serpentine is similar to that at Slishwood, but it is slightly darker in colour and shows no banding nor chrysotile veins.

(3) *Stonepark*. (6" Leitrim No. 14/1.2). Serpentine here occurs in a belt running E.S.E.-W.N.W. along the edge of an alluvial flat. The rock is exposed over a length of 400 m., and passes under thick alluvium to east and west. Two traverses were run across this belt and showed its width to be about 100 m. The

serpentine resembles that at Slishwood, but is somewhat darker in colour and less talcose. It shows similar pseudo-bedding, but no well-defined banding is evident.

(4) *Pollboy* (6" Leitrim No. 11/1) Here a narrow belt of serpentine lies between gneiss and Carboniferous limestone. It is divided from the latter by a fault with which appears to be associated a copper mineralization in the limestone. The serpentine runs in a north-east to south-west direction, is about 450 metres long, and is cut off by faults at each end. Its width as shown by two traverses is about 70 metres. The rock is similar in appearance to that at Stonepark and Aghamore.

(5) *Clew Bay Area*. (6" Mayo Nos. 87, 88. 1" No 74) No magnetic observations have been made here. The analyses are given in a succeeding paragraph; the samples were taken from a chain of occurrences extending twelve miles from east to west. These are steep-sided dykes with a certain degree of continuity, intrusive into gneisses, schists, and quartzites of the Croagh Patrick range.

ANALYSES OF SERPENTINES.

| No. | Locality | Nickel as NiO. | | Total Iron as Fe_2O_3 | | vt. Magnetite |
|-----|--|-------------------|-------------------------------|---------------------------------------|---|---------------|
| | | %* | | %* | | % |
| 1. | Slishwood, see 25" map | 0.30 | av : 0.257 = 0.202% Ni. | 8.5 | av : 8.1 = 7.8 Fe_3O_4 | 8.2 |
| 2. | Slishwood ... | 0.26 | | 7.9 | | 7.6 |
| 3. | Slishwood ... | 0.08 [†] | | 7.8 | | 7.5 |
| 4. | Slishwood ... | 0.26 | | 7.7 | | 7.4 |
| 5. | Slishwood ... | 0.26 | | 8.3 | | 8.0 |
| 6. | Slishwood ... | 0.29 | | 8.0 | | 7.7 |
| 7. | Slishwood ... | 0.26 | | 7.8 | | 7.5 |
| 8. | Slishwood ... | 0.24 | | 8.0 | | 7.7 |
| 9. | Slishwood ... | 0.28 | | 11.1 | | 10.7 |
| 10. | Pollboy ... | 0.34 | | 6.3 | | 6.05 |
| 11. | Aghamore ... | 0.26 | | 8.0 | | 7.7 |
| 12. | Stonepark ... | 0.26 | | 8.0 | | 7.7 |
| 13. | Clew Bay area, Co. Mayo (exact locality un- defined) ... | 0.21 | | 8.0 not included in average. | | |

| No. | Locality | Nickel as NiO. | | Total Iron as Fe ₂ O ₃ | vt. Magnetite |
|----------------|----------|----------------|-----------------------------|---|---------------|
| | | %* | | %* | % |
| 14. Mayo 88/1. | 1a. | ... 0.36 | av : 0.298 =0.235%Ni. | 7.0 | 6.7 |
| 15. | 1b. | ... 0.20 | | 7.5 | 7.2 |
| 16. | 1c. | ... 0.20 | | 6.9 | 6.6 |
| 17. | 1d. | ... 0.20 | | 8.3 | 8.0 |
| 18. Mayo 88/3 | 2a. | ... 0.26 | | 7.6 | 7.3 |
| 19. | 2b. | ... 0.36 | | 8.5 | 8.2 |
| 20. Mayo 87/4 | 3a. | ... 0.46 | | 9.7 | 9.3 |
| 21. | 3b. | ... 0.20 | | 12.8 | 12.3 |
| 22. | 3c. | ... 0.29 | | 9.3 | 8.9 |
| 23. Mayo 87/4 | 4a. | ... 0.42 | | 6.7 | 6.4 |
| 24. | 4b. | ... 0.24 | | 7.2 | 6.9 |
| 25. | 4c. | ... 0.20 | | 5.6 | 5.4 |
| 26. | 4d. | ... 0.39 | | 9.0 | 8.65 |
| 27. | 4e. | ... 0.39 | | 7.6 | 7.3 |

* Analyst : The State Laboratory. A preliminary determination of nickel on samples 1-12 in the undried condition was also made by Mr. T. Murphy (G.S. Laboratory) who found values between 0.15% and 0.27% NiO average 0.201% NiO, which is in good correspondence with the State Laboratory figures for dried samples.

† 0.08 checked by repetition.

SUMMARY AND COMMENTS.

Chemical Composition. The serpentines which form the subject of this short investigation would not, in the light of modern petrology, be held to be anything but dykes derived from some ultra-basic parent, and intruded into the metamorphic complex which is here almost certainly pre-Cambrian. The contact of the Slishwood serpentine with the Lower Carboniferous rocks which overlie it, has not been adequately exposed, but its pre-Carboniferous age is inferred.

The average content of nickel in the Sligo-Leitrim group of serpentines is 0.202%, with 8.1% total iron estimated as Fe₂O₃ equivalent to 7.8% magnetite. The Mayo group contains a little more nickel, 0.235% average, while the iron content calculated as magnetite is also 7.8%, the average of 14 samples being identical with that of 12 from the Sligo-Leitrim group. Individual assays do not deviate very much from the mean in most cases.

It is improbable that all the iron is present as magnetite, but the evidence of thin sections and magnetic separation suggests that most of it is in this form. If so, the content in 12 samples from Shishwood ranged between 6.05% and 10.7% as against Hardman's 2% to 15 %, the individual content being close to 8% in most cases. The Curie point corresponding to magnetite has kindly been determined by Dr. J. H. J. Poole.

It has nowhere been possible to confirm the high contents of nickel reported by Hardman, but no specimen has been found which does not contain nickel. This may be due to the greater reliability of the modern dimethyl-glyoxime analysis, which is specific for this metal. A sampling error may however occur through using weathered specimens in which the nickel tends to become slightly concentrated.

It is interesting to note a parallel example in the early work of Bonney (1891) on the Lizard Serpentes (Cornwall), which indicated up to 0.6% Ni; this was subsequently reduced by Flett and Hill to the lower figure of 0.15%. The content of 0.2% Ni given in our Irish analyses compares with those from the Cornish area, and with those cited by Clarke (*Data of Geochemistry, U.S.G.S.*). A similar occurrence has recently been described from Dillon, Montana,—a low grade nickel silicate deposit in which the nickel content of serpentine and related rocks ranges between 0.15% and 1.25%. In the case of the serpentes of New Caledonia, Blanchard (1944) has pointed out that the average content of nickel, judged from samples taken all over the island regardless of proximity to commercial deposits, is 0.3%.

The Irish analyses shows a fair degree of uniformity in total nickel and iron content. The Mayo group is derived from a more or less continuous chain of serpentine dykes twelve miles in length, and a distance of 60-70 miles intervenes between these and the Sligo-Leitrim group at the north eastern extremity of the Slieve Gamph range of hills.

No appreciable quantity of sulphide or arsenide minerals has so far been detected in this preliminary investigation; the magnetite fraction obtained by pulverising the rock and by magnetic separation does not appear to carry nickel, which only becomes soluble on fusion of the rock with alkali. This seems to show that it was present as a primary silicate in the ultra-basic parent rock, as in the occurrence of New Caledonia, the Urals, and Dillon, Montana.

There is no evidence as yet that would indicate the presence of the highly magnetic oxide of iron and nickel, Trevorite, which was described from South Africa in 1921, or of the suggested new nickel silicate allied to talc which is the subject of a paper by the late F. C. Partridge (*Trans. G.S. S. Africa*, 46, 1943); but it would be well to bear these possibilities in mind in future work. The possible occurrence of chromite has not been studied.

There is some confusion in Hardman's original paper regarding the asbestos veins which are termed 'chrysolite' or tremolite,' although Hull accepted the latter determination very doubtfully. This asbestos is more correctly, chrysotile, as Kilroe points out in G. S. Memoir 55; but comparatively few of the bands exposed at the surface contains fibres of commercial flexibility or length.

From the economic point of view, the concentration of nickel and magnetite in these rocks is not sufficient to admit of their commercial recovery by present-day processes. The Orsk smelter of the Urals appears to handle an average ore containing in the undried condition as little as 1% Nickel; the average slag loss has been given as 0.25% and sometimes as low as 0.15%. The New Caledonian nickel-silicate ore may have a metal content of 5%, but slag losses range from 0.50-0.60% nickel. In the Ural plants the ore is smelted in blast furnaces with gypsum and other fluxes to form a matte. If it had been possible to sustain Hardman's figures, the extraction of Irish nickel on these lines might have been considered; but the average nickel content of the Slieve Gamp serpentine appears to be approximately equal to the slag loss at the Orsk smelter. The results of investigations carried out by the Anaconda Copper Co. on the comparable but somewhat richer deposits at Dillon, Montana, have not been disclosed.

Magnetic characteristics of the Sheshwood dyke.—Some magnetite is disseminated in the gneiss bordering the serpentine, but a survey of the vertical component of intensity over this body shows:—

- (a) a general anomaly about 1,000 γ higher than in the surrounding country, and
- (b) a number of very localized erratic anomalies of extremely high intensity without regard to sign. These are not related to the quantity of magnetite present, but rather to the orientation and polarity of the magnetic units which are scattered throughout the rock.

Mr. T. Murphy, the physicist attached to the Survey, observes that the general low anomaly over the serpentine, so far as could be ascertained, is in accordance with the earth's field. Hence it is inferred that this anomaly was produced solely through induction by the earth's field in material whose susceptibility was greater than that of the adjoining rocks. This susceptibility is not known, hence a comparison between theoretical and practical results can not be made.

The very local readings of great intensity, which are definitely referred to polarization, appear to be due to material close to the surface. With one or two exceptions all occur where the overburden is very thin, and the majority near a cliff-face or upjutting outcrop. The exceptions are covered by drift of undetermined thickness.

Mr. Murphy suggests that magnetite in the original dyke would, on cooling below the Curie point of about 600°C, acquire a polarity in accordance with the earth's field then prevailing. In this form the coercive force would be quite low and the rock as a whole would show little or no polarity (It is clear, however, that the molecular regrouping caused by the subsequent hydration of an original peridotite to serpentine would have unpredictable effects). It is also suggested that the strong local polarization occurring chiefly near the highest points of the serpentine may have been produced at a much later period by the intense fields of lightning flashes. The general anomaly of course persists well beneath the cover of Carboniferous rocks at the south extremity of the dyke whose ultimate length is unknown. There appear to be too many factors unknown at present, to permit of a convincing explanation.

I am indebted to the State Chemist for the determinations of nickel and iron in the foregoing analyses. The variometer observations, geological field work and associated laboratory investigations were carried out by Mr M. A. Cunningham of the Geological Survey and Mr. T. Murphy of the Emergency Research Bureau who kindly provided the variometer. Permission to reproduce Ordnance Survey maps on which the work was based, and to publish this paper, is by courtesy of the Ministers for Finance and for Industry and Commerce, respectively.

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No 15.

THE MAGNETIC PROPERTIES OF THE SERPENTINE DEPOSITS IN THE SLISHWOOD GAP, COUNTY SLIGO, IRELAND

By

W. E. SEEDS, M.Sc. AND J. H. J. POOLE, Sc.D.

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PLATE II

INTRODUCTION

DURING 1942 and 1943 the Geological Survey of Ireland undertook a partial magnetic survey of the serpentine deposits near Slishwood, County Sligo, Ireland, since it has been known for some time that the serpentine deposits of County Sligo and County Mayo contain a small percentage of nickel, which might in the future have commercial possibilities, (1) The survey was conducted with a Watt's Vertical Variometer. Very large and rapid variations in the vertical magnetic field were observed, and the nature of these variations indicated that the serpentine must be fairly strongly permanently magnetised, the direction of magnetisation being sometimes nearly in the opposite direction to the existing Earth's field. Tests with a magnetometer on hand specimens confirmed this hypothesis, and showed that specimens from different localities differed greatly in intensity of magnetisation.

It was accordingly decided to conduct more accurate measurements on the magnetic properties of the serpentine, using specimens whose original position and orientation were known. It was hoped by this means to gain some knowledge of the nature, magnitude, and direction, of the original polarising magnetic field. As the number of oriented specimens was limited, some tests were also made on unmarked specimens.

The nature and general characteristics of the Slishwood serpentine is discussed in a recent paper by D. W. Bishopp, Director of the Irish Geological Survey (1). A sketch map (Fig. 1.) showing the geological character of the district and the locality from which the specimens were obtained is reproduced from this paper. The ser-

pentine deposits consist of a narrow belt varying in width between about 150 and 110 metres, and about 2500 metres in length. At the northern end, the outcrop disappears under the waters of Lough Gill, and at the southern end under the Carboniferous limestone. Magnetic observations have shown that the serpentine extends for at least another 400 metres southward below this Carboniferous cover, which presumably indicates that the serpentine is at least pre-Carboniferous in age. It may be remarked that the serpentine belt now lies at the bottom of a steep-sided stream valley, and this fact introduces some difficulty in explaining the origin of the permanent magnetisation of the serpentine, as will be seen later.

EXPERIMENTAL ARRANGEMENT.

The apparatus, used for determining the intensity and direction of the original permanent magnetisation of the serpentine, is shown schematically in Fig. 2. It

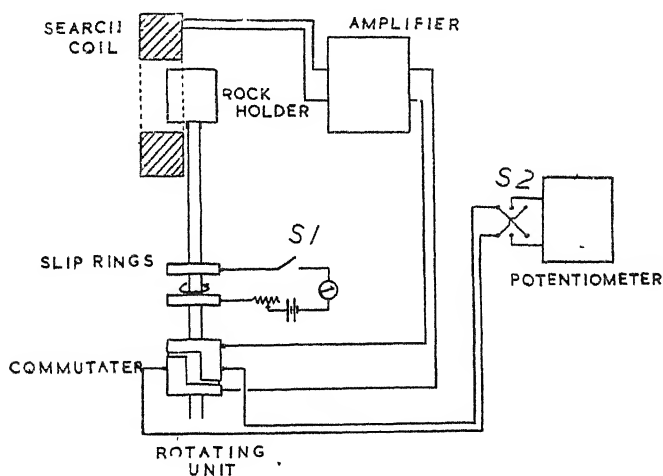


Fig. 2.

is a simplified version of Johnson and McNish's instrument, but by the addition of the current coil round the specimen holder, a rough standardisation of the instrument can readily be obtained, and also the susceptibility of the specimen up to fields of about four oersteds can be determined. A small cubical specimen, whose orientation is known, fits in the rock holder, which is rotated in front of a fixed coil of a large number of turns of fine copper wire. The rock holder consists of a rectangular copper box with a break in the copper at one corner, so that no current can flow round the box. The vertical ends of the box are open to enable the specimen to be easily inserted and removed. By altering the position of the specimen in the box, its magnetic moment about three perpendicular axes can be determined, and hence the magnitude and direction of its total magnetic moment calculated. The standardising direct current coil is wound round the outside of this box, the direct current used for exciting the coil being led in through two slip rings in the lower

part of the instrument. The alternating voltage induced in the fixed coil is first amplified by a two or three stage low frequency amplifier, and then rectified by a synchronous commutator. It was found that for the small voltages concerned a metal-oxide rectifier was not suitable. The amplified rectified output voltage is measured with a Leeds Northrop potentiometer. This instrument gives a very wide range of accurate voltage measurement. The insertion of a 12 μ f. condenser in parallel with the output brushes of the commutator was found to be advantageous, as it led to a considerable steadying effect in the readings of the potentiometer.

The details of the rotating unit are shown in Fig. 3 and Plate 11. A vertical brass driving shaft is carried in two white metal bearings, which are fixed in two aluminium disks. These disks are rigidly connected to each other by four aluminium pillars, the structure being stiffened by two parallel vertical ebonite plates, which also carry the brush gear. These plates are fixed with screws to flat faces on the vertical aluminium pillars. The driving shaft carries at its lower end a pulley, which is driven by a belt drive from a single phase $1/8$ H.P. induction motor placed about 7 ft. from the instrument. It is more convenient to run this motor with its axis of rotation horizontal, and this can easily be arranged by the use of two small jockey pulleys, which can also be used for adjusting the belt tension. Since the load on the motor is small its speed will be very nearly constant. The belt is made of good quality twine about $1/16$ inch diameter, the two ends of the twine being spliced together, as any form of leather belt with a fastener was liable to produce vibration. To eliminate belt slip a dressing was used.

The commutator and slip ring system is mounted on the rotating shaft between its bearings. Above the upper bearings, the shaft carries a brass disk to which is attached a tapered ebonite column. The rock holder is fixed to a small diameter vertical brass tube carried from the top of the ebonite column. The standardising coil wound round the rock holder is fed by two leads up this tube from the slip rings, as shown in diagram. It is possible to change the orientation of the rock holder relative to the commutator by rotating the brass tube in its holder, which is fixed to the top of the ebonite column. The tube can be fixed in any position by means of a small brass grub screw in this holder. It may be pointed out that owing to the overhang of the ebonite column, the alignment of the various components must be very accurate, otherwise undue vibration will occur. This can be best achieved by doing the final turning and boring of the ebonite column after it is fixed to its brass base plate. The upper bearing plate can be used as a steady during this operation, the lower bearing surface of the brass driving shaft being carefully gripped in the lathe chuck. A final true up can now also be given to the commutator and slip ring surfaces.

The design of the brush gear gave a good deal of trouble. Various forms of copper and carbon brushes were tried. The final form of brush adopted consisted of a short length of 22 S.W.G. copper wire pressed against the commutator by means of a phosphor bronze single leaf spring. The copper wire was soldered to the end of the spring, and made contact on one side near its outer end with the commutator. The pressure on the brush could be adjusted by means of a screw which pressed on the leaf spring about $3/4$ inch from its anchorage. These brushes, if kept lubricated, were found to function satisfactorily.

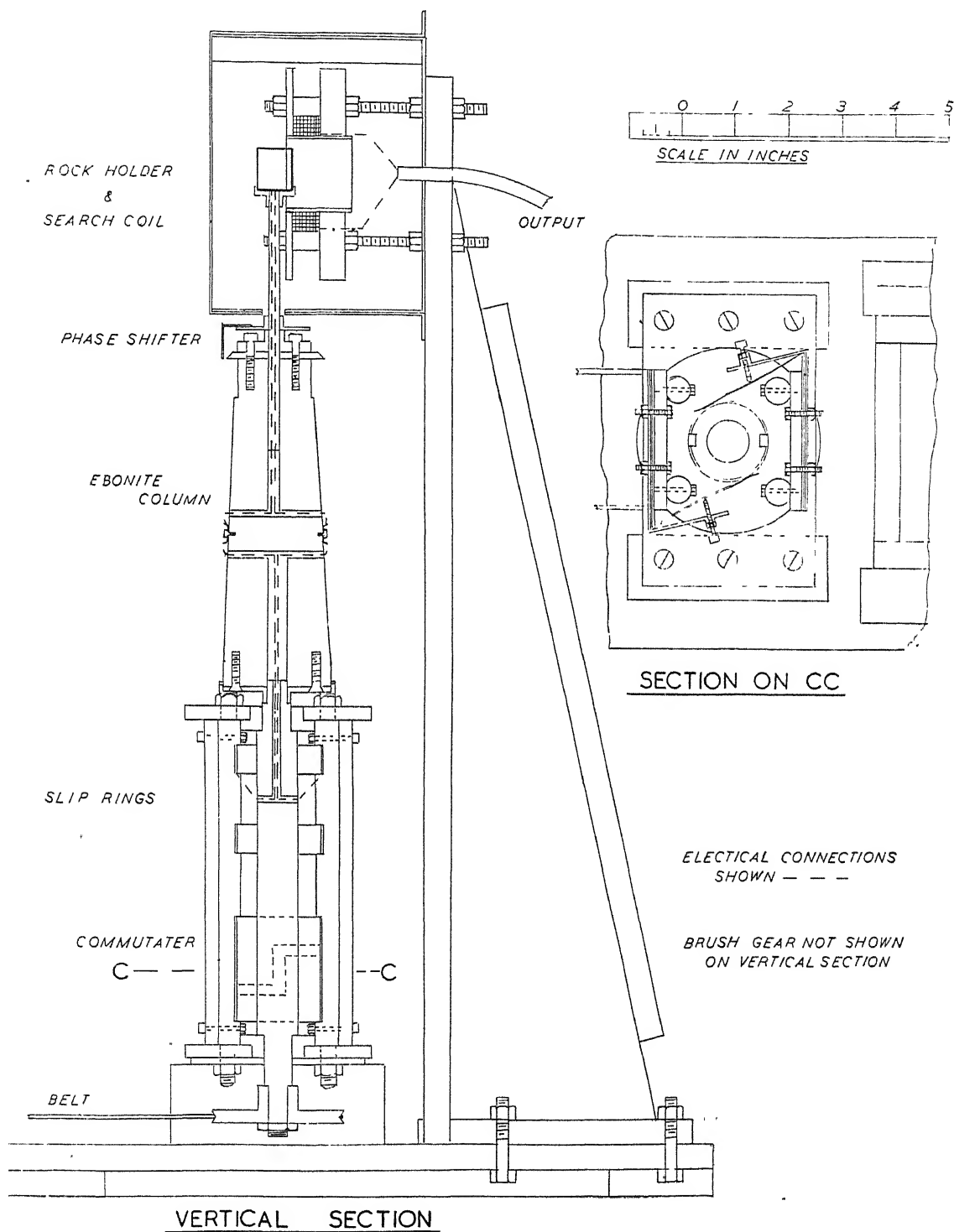


FIG. 3.

The standardising current coil wound round the box shaped holder contains about 70 turns of 40 S.W.G. enamelled copper wire, the winding being distributed over nearly the full length of the box. The exciting D.C. current is fed in through the slip rings, as already mentioned. To reduce the effect of any irregular change in the resistance of the slip ring contacts it is advantageous to have a resistance of at least 1,000 ohms in series with the D.C. Voltage supply. The polarity of the current coil was determined by the use of a small compass needle, and the rock holder and switches marked accordingly. The polarity of any axis of a specimen cube relative to the marked end of the rock holder is given by the direction of the reversing switch S_2 Fig. 2 when a balance is obtained on the potentiometer.

The fixed search coil, in which the A.C. voltage is generated by the rotation of the rock specimen or of the standardising current coil, consists of about 4,000 turns of 40 S.W.G. enamelled copper wire wound on a hollow ebonite spool, and mounted on a rigid wooden column fixed to the base of the instrument. The search coil is mounted in such a way that its position relative to the rotating specimen holder can easily be adjusted. The coil and rotating holder are enclosed in an aluminum screening box. In setting up the instrument the plane of the search coil should be perpendicular to the magnetic meridian, since in this position the Earth's horizontal magnetic flux through the coil is a maximum and consequently any small torsional vibration of the coil about its vertical axis will produce the minimum E.M.F. in the search coil.

The output from the search coil is taken through a screened lead to the amplifier. It is advisable to have the amplifier, potentiometer, etc., mounted on a separate bench from the rotating unit to reduce any effect of vibration. For the same reason the motor should be carried on rubber blocks. It was found convenient to arrange that either the A.C. output from the amplifier or the rectified output from the commutator could be fed to a cathode ray oscillograph if desired. This showed that all leads must be carefully screened in order to avoid 50 cycle A.C. pick up. The frequency generated by the rotating unit was about 22 cycles per second, and was nearly a pure sine wave.

CALIBRATION OF INSTRUMENT

A constant current of about 20 milliamps is first passed through the rotating coil, and the orientation of this coil relative to the commutator adjusted to give the maximum reading on the potentiometer. Since the magnetic moment of the coil will be approximately along the axis of the coil, if a rock specimen is now placed in the coil and the current cut off, the magnetic moment of the specimen in the same direction may be measured. By suitable rotation of the specimen in the holder the three mutually perpendicular components of its magnetic moment can be determined.

By varying the current in the rotating coil the sensitivity of the apparatus may be now investigated, the rock specimen being removed from the holder. This showed that the relation between coil current and rectified output voltage was linear within about 2% up to the maximum current used, about 40 milliamps. The sensitivity can be most easily expressed in output volts per milliamp of coil current,

For the two-stage amplifier this figure was 0.030 volt per milliamp., and for the three-stage 0.67 volt per milliamp. These values can be rapidly checked at any time.

We can now obtain an approximate value for the intensity of magnetisation of a specimen. We must assume that the distribution of the field of the current coil is similar to that of the rock specimen inserted in the holder, when the component of magnetisation of the specimen along the axis of the holder is alone considered. The magnetic moment of the coil

$$M = ACn/10 \text{ C.G.S. units (nearly)}$$

where A = cross sectional area of coil in cm^2

C = exciting current in amperes

n = number of turns in coil

Inserting the known values of A and n , we find that the magnetic moment of the coil per milliamp is 0.020 C.G.S. unit. The output voltage per milliamp, exciting current is, however, known, and hence we can calculate the magnetic moment required to give 1 volt output. The final figures are

Magnetic moment per unit output voltage:

2 stage amplifier = 0.68 C.G.S. units

3 stage amplifier = 0.030 C.G.S. units

It is clear, therefore, that if the output voltage for a given specimen is determined, its component of intensity of magnetisation along the axis of the coil is I_x , say, where

$$I_x = \frac{\text{Output voltage} \times \text{magnetic moment per unit output voltage}}{\text{volume of cubical specimen}}$$

MEASUREMENT OF SUSCEPTIBILITY OF SPECIMEN.

It is also possible to use the instrument to measure the susceptibility of the specimen up to fields of about 4 oersteds. To do this we plot the output voltage against the exciting current when the specimen is in the holder. In this case also a linear relation is obtained, showing that for the fields used, the susceptibility of the specimen was sensibly constant. The slope of this line is however, slightly greater than that obtained in the standardising run when the holder is empty. We cannot, however, determine the permeability of the specimen from these two curves only. The external magnetic field in which the specimen is placed can be approximately calculated from the known dimensions of the current coil and the value of the exciting current, but owing to the cubical shape of the specimen the depolarising effect of its ends is considerable. The problem can be considered as follows.

The output voltage will be proportional to the total flux of B through the coil. We can apply the usual magnetic circuit equation;

$$N = \frac{4\pi}{10} \cdot \frac{\text{ampere turns}}{\text{magnetic reluctance}}$$

where N = total flux of B ,

The magnetic reluctance of the circuit can be considered to consist of two parts, i.e. the reluctance of the path inside the current coil (Q_2) and the reluctance of the return path outside the coil (Q_1). If Q is total reluctance,

$$Q = Q_1 + Q_2$$

If we fill the rock holder with stalloy sheets, the value of Q_2 will be very small compared to Q_1 owing to the high permeability of stalloy even for small fields. From this it follows, that very approximately ;

$$\frac{Q_1 + Q_2}{Q_1} = \frac{\text{Output voltage per milliamp for stalloy sheets}}{\text{Output voltage per milliamp for air.}}$$

The experimental values gave the relation

$$Q_2 = 1.66Q_1$$

Consider the coil now filled with a rock material of permeability μ where μ is small.

Then the total reluctance $Q' = Q_1 + \frac{Q_2}{\mu}$.

$$\begin{aligned} \frac{\text{The output voltage per milliamp for rock}}{\text{The output voltage per milliamp for air}} &= \frac{Q}{Q'} = 2.66 \left/ \left(1 + \frac{1.66}{\mu} \right) \right. \\ &= \frac{2.66\mu}{\mu + 1.66} . \end{aligned}$$

This quantity can be determined experimentally, call it z ;

$$\text{so that } z = \frac{2.66\mu}{\mu + 1.66}$$

$$\text{or } \mu = \frac{1.66z}{2.66 - z}$$

$$\text{and the susceptibility } k = \frac{\mu - 1}{4\pi} .$$

The actual results given in this paper may be divided into two classes, i.e. (a) the determination of the magnitude and direction of the original intensity of permanent magnetisation of the available specimens and the measurement of their susceptibilities, and (b) some further investigations on the properties of the serpentine.

ORIGINAL PERMANENT MAGNETIZATION AND SUSCEPTIBILITIES OF SPECIMENS

The number of oriented specimens that could be obtained was small. This was due both to the very small number of surface exposures of the serpentine, and also to the difficulties of transport during the war years, which made the collection of further specimens impracticable. Only five oriented specimens were available, and it was found impossible to cut a satisfactory cube out of one of these, as the rock always broke before the cube was completed. The value of the original total inten-

sity of magnetisation, its direction, and the susceptibility of each specimen are given in the Table. The locality of the specimen is indicated by a number (See Fig. 1.)

TABLE

| Specimen Number | Intensity of Magnetisation | Direction of Total Magnetisation | | Susceptibility |
|-----------------|-----------------------------|----------------------------------|---------------|-----------------------------|
| | | Dip | Declination | |
| 7A | 17.9×10^{-4} C G S | 46° | 104° E | 2110×10^{-6} C G S |
| 7B | 58.0×10^{-4} | 38° | 50° E | 2380×10^{-6} |
| 6B | 490×10^{-4} | — | — | 1580×10^{-6} |
| 5 | 17.4×10^{-4} | -2° | 72° W | 1770×10^{-6} |
| 4 | 0.6×10^{-4} | 13° | 179° E | Too small to measure |
| 2 | 255×10^{-4} | — | — | 3160×10^{-6} |

The declination is given in degrees measured either east or west of true north

The results show the wide variation in the original intensity of magnetisation of the specimens. The differences in susceptibility are not nearly so marked, and agree with previously determined values for serpentine, for which the accepted values of the susceptibility have a very large range (3). The direction of the total magnetisation seems to be quite erratic, and would seem to bear no relation to any fixed direction of the Earth's field. It may be noted that the field of about 3 oersteds used in measuring the susceptibility of the specimens produced no permanent effect on their residual magnetism, which would also lead us to conclude that the permanent magnetism of the specimens can not have been produced by the Earth's field, once the serpentine had cooled. Specimens 7A and 5 gave rather different values for the susceptibility along different axes, the minimum susceptibility being about two-thirds of the maximum. The figures given in the table are mean values. This effect was not shown by the other specimens, 7B, 6B and 2, but one of the first unmarked specimens used also showed it. Specimen 4 seems anomalous since it shows neither permanent magnetisation nor measurable susceptibility. An Ultropak microscope was used to examine the surfaces of the cubical specimens. This showed that apparently there exists some relationship between the number of unchanged olivine crystals visible on the surface of the specimen and the intensity of magnetisation. Thus 6B and 2 had large numbers of crystals visible, while 7A, 7B, and 5, all had few visible crystals, whereas 4 had none. This apparent connection may be accidental, as the number of specimens is so small, and no theoretical justification can be advanced for it.

SOME FURTHER INVESTIGATIONS ON THE PROPERTIES OF THE SERPENTINE.

Figure 4 gives the results of placing the various specimens in a demagnetising field for a standard period of one minute, and then measuring the residual permanent magnetism. The value of the demagnetising field in oersteds is plotted horizontally

and the rectified output voltage of the amplifier vertically. It will be seen that in all cases a considerable field is necessary to destroy the original magnetisation of the

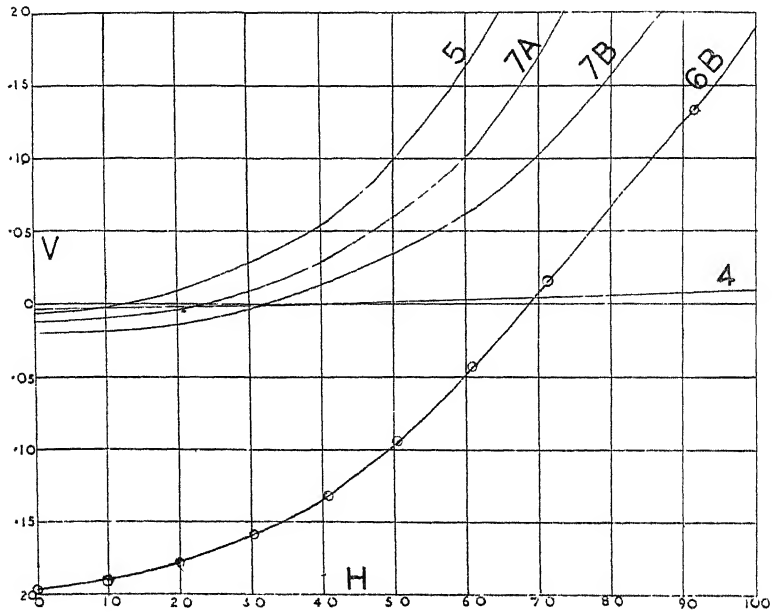


FIG. 4.

specimen, and that this field increases with the magnitude of the original magnetisation. For specimen 5, the weakest normal specimen, the value of the completely demagnetising field, is about 13 oersted, whereas for 6B, the strongest, the value

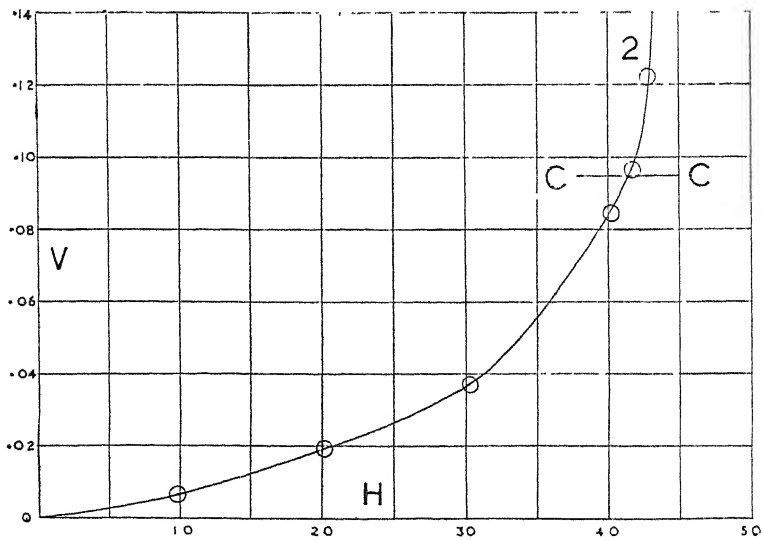


FIG. 5.

is about 69 oersted. It may, I think, be safely concluded that fields at least as large as these would have been required to produce the original magnetisation, if the serpentine was at ordinary temperature.

To investigate this point further specimen 2, which was originally approximately half as strong as 6B, was completely demagnetised by the use of a gradually decreasing alternating magnetic field. The results of placing this specimen for the standard period of one minute in a gradually increasing field and measuring the resulting permanent magnetism are shown in Fig. 5. The horizontal line CC gives the original value of the magnetisation of the specimen, and it will be seen that it takes a field of 43 oersted to bring the demagnetised specimen back to this value, which agrees with the results shown in Fig. 4. For these experiments a standard solenoid was

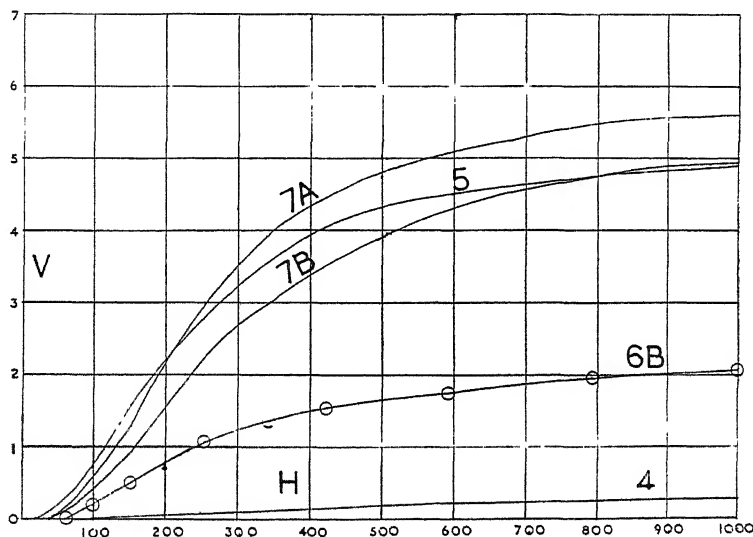


FIG. 6.

used to produce H, and the actual field acting on the specimen would be slightly less than the plotted values owing to the depolarising effect of the cubical specimen. Owing to the low susceptibility of the specimen this effect would be small.

Fig. 6 gives the results of placing the specimens in fields up to 1,000 oersted. For these fields an electro-magnet, which had been standardised by means of a Grassot Fluxmeter, was used. The curves show that specimens 7, 5, and 7B are very similar, and are all capable of being fairly strongly magnetised. Thus for 7B the value of the permanent magnetism produced by 1,000 oersted is more than 200 times the original magnetisation of the specimen. 6B, the strongest original specimen, differs slightly, since its value at 1,000 oersted is only about 40% of theirs, and the ratio of its original to final value is only about 10/4 is, however, still quite anomalous, and even 1,000 oersted produced very little permanent magnetism. The explanation of this peculiarity is obscure, since the chemical analyses given by Bishopp (*loc. cit.*) show that the serpentine is fairly uniform in total iron content,

and it can only be suggested that in some cases the percentage of total iron present as magnetite is small. It may be remarked that there is no obvious difference in appearance between specimen 4 and the other specimens, but there is no doubt as to its difference in magnetic properties.

The effect of heat on the magnetisation of the serpentine was also investigated. A cube, which had been placed in a strong magnetic field, was heated for about 15 minutes at gradually increasing temperatures in a small electric furnace, and its residual magnetism after cooling measured. It was found that a temperature between 570°C and 600°C reduced the permanent magnetism to a very low figure. This would agree with the view that magnetic properties of the serpentine are due

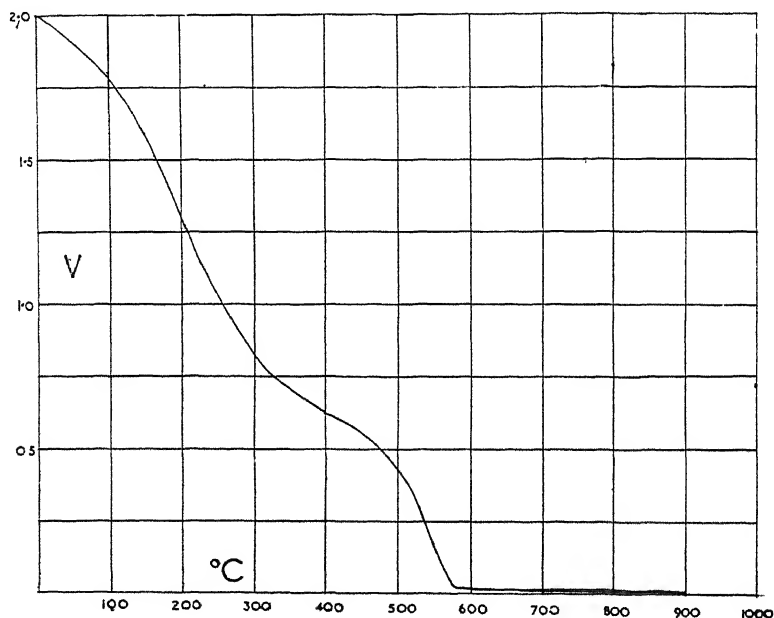


FIG. 7.

to the presence of magnetite, since the Curie Point of magnetite is about 585°C. The results are illustrated in Fig. 7. There is a distinct concavity in the curve centred about 350°C, and it might be concluded that this is due to the presence of nickel (Curie Point 374°C), but since the nickel is nearly certainly present as a salt, this idea seems rather improbable. It is hoped to investigate this question more fully. It may also be noted that the permanent magnetisation produced in the specimen by cooling from above the Curie Point to air temperature in the Earth's field, is about equal to that of Specimen 5, and hence is only a small fraction of the original permanent magnetisation of Specimens 6B and 7.

Some measurements on the electrical resistivity of the serpentine were also made. Its value was found to vary from 10^4 to 2×10^5 ohms per cm. cube in two directions at right angles in a single specimen, but no connection between the resistivity along

a given axis of a specimen and the magnitude of the original permanent magnetisation in the same direction could be established

The lower figure of 10^4 ohms per cm. cube is about $\frac{1}{4}$ th of the value found by Eve and Keys, but such variations in natural products are not surprising. The main point of interest is that the resistivity of the serpentine is much lower presumably than that of the gneiss which surrounds it, whose resistivity probably lies between 10^7 and 10^8 (4). The simple method we used for measuring the conductivity of the serpentine by observing with a micro-ammeter the current driven by 200 volts D.C. through the cube, was not sufficiently accurate to measure the resistivity of a basalt cube, which showed that its resistivity was greater than 10^7 ohms per cm. cube. It is doubtful, however, if the difference in resistance between the serpentine and the gneiss, measured under laboratory conditions, would be of any importance in the field, where both strata must be completely saturated with surface water. It is conceivable, nevertheless, that the greater observed conductivity of the serpentine might lead to a concentration of lightning flashes to it.

DISCUSSION OF RESULTS

The conclusions that may be drawn from our results may be briefly summarised as follows.

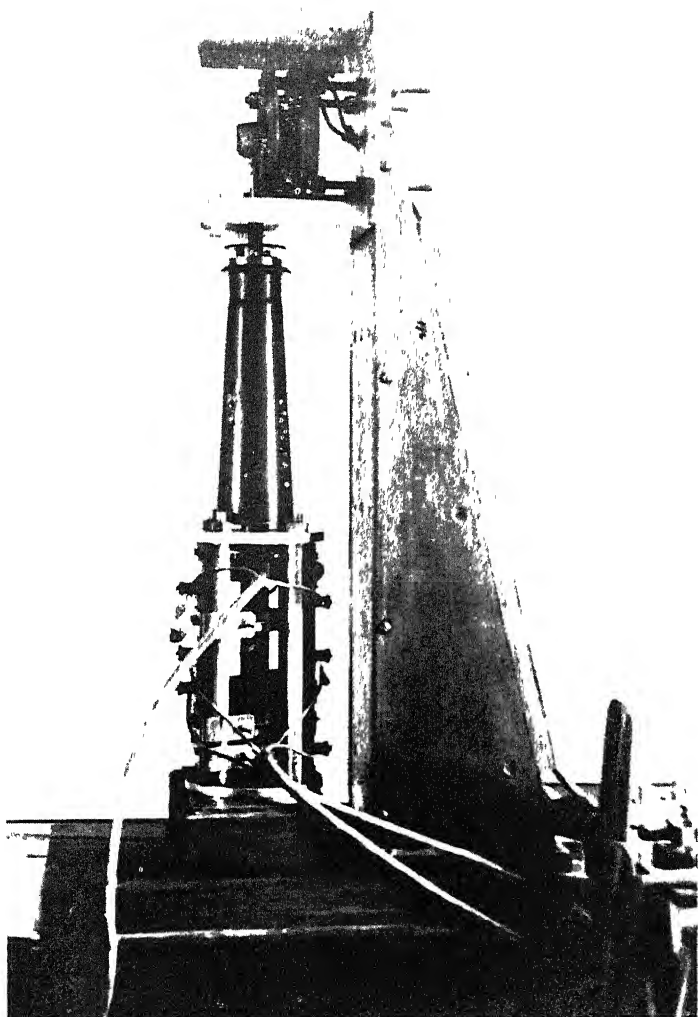
- (1) The magnetic properties of the serpentine are due to the presence of magnetite.
- (2) The general magnetic anomaly observed in the area is produced by the high susceptibility of the serpentine.
- (3) The intensely local very large variations observed in the surface magnetic field are due to certain portions of the serpentine being fairly strongly permanently magnetised.
- (4) The uncertain distribution both of the direction and magnitude of this permanent magnetism indicates that it cannot be attributed to the action of the Earth's field.
- (5) The magnetic properties of the weakly magnetised specimens and the stronger ones are very similar. This indicates that the difference in their permanent magnetism must be attributed to the original polarising magnetic field. Specimen 4 is however anomalous.
- (7) It seems probable that this field must have been produced by lightning flashes. Norinder (5) has shown that the horizontal magnetic field produced by a lightning discharge may be as large as 5,000 oersted, an average value being about 1,000 oersted. The average duration of a flash is below 0.005 sec. and this might reduce its magnetising effect on the serpentine. Our results however show (Fig. 4) that a field of the order of 100 oersted if applied for a long time would be sufficient to produce the maximum permanent magnetisation found. It is well known that links made of a cobalt-nickel iron alloy, of high

magnetic retentivity, attached to steel power line pylons can be permanently magnetised by lightning discharges to the pylons. This fact had been used both in the United States (6) and Germany (7) to estimate the current passing through the pylon. We may therefore safely conclude that lightning flashes would be capable of producing the permanent magnetism of the serpentine. This view would also explain the erratic distribution of this permanent magnetism. The main objection to the lightning hypothesis is the topographical nature of the district. The Serpentine now lies at the bottom of a steep-sided stream valley, (Fig 1). We should consequently imagine that lightning discharges to it would seldom if ever occur, but the behaviour of lightning is so peculiar that it is rash to put too much weight on this fact.

In conclusion we would like to express our thanks to Mr. Bishopp and Mr. Murphy of the Geological Survey of Ireland for the supply of the necessary specimens, and to the Ordnance Survey of Ireland for permission to publish Fig. 1 which is based on the 6 inch Ordnance Sheet of the Shishwood area.

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No. 16.

AWARD OF THE BOYLE MEDAL TO THE LATE PROFESSOR
THOMAS J. NOLAN, D Sc , F.R.I.C.†

REPORT OF THE COMMITTEE OF SCIENCE AND ITS INDUSTRIAL APPLICATIONS

THE late Professor Nolan was well-known for his distinguished work in organic chemistry and its technical applications; he had also a wide knowledge of, and experience in, general analytical work. As might be expected he had therefore an exceptionally good basis for his chemical teaching. In his later years he contributed valuable papers to the Proceedings of our Society, but these constitute but a minor portion of his life-work in chemistry.

Born in Dublin in 1888 Nolan graduated with first-class honours in chemistry and physics in the Royal University. After some teaching work he was awarded a travelling studentship for his thesis on "The higher ketones and secondary alcohols derived from the amides of palmitic and stearic acids" This was published in the Proceedings of the Royal Irish Academy His work in Geneva with Pictet was not completed owing to the illness of the latter, and he then worked in London under Prof. Smiles on the "Isomerism of the sulphides of β -naphthol," five publications by the Chemical Society in 1912 and 1913.

In 1913 Nolan worked under Zincke in Marburg, and published in Liebig's *Annalen* "Salpetersäure-chinitrol aus 3 . 5 . 6 trichlor-o-Cresol und Umwandlungsprodukte." He was later associated in Berlin, with the great Willstätter, who was then engaged in his classical researches upon the pigments of plants. Nolan isolated the colouring matters of the rose and the paeony in a pure condition, and established their constitutional formulæ.

In 1914 Nolan returned to Dublin and was awarded the Doctor of Science degree of the National University of Ireland The next year he joined the Research Staff of Messrs. Nobel's Explosives Co. and worked on nitrobodyies. When the firm started a "Fine Chemicals Department" Nolan developed the manufacture of a number of organic compounds. He later worked on propellant powders, and developed the production of a new one made without the aid of a volatile solvent. This "Ardeen Cordite" was adopted as a Service powder.

† The Medal was awarded by the Council on November 1st, 1945. It was presented by the President to Mrs. Nolan at the stated General Meeting of the Society on December 6th, 1945.

In 1919, when various explosives companies were amalgamated, Nolan was placed in charge of development work on propellants, and devised several new types of sporting powders.

In 1924 Imperial Chemical Industries appointed Nolan to investigate difficulties in the manufacture of tetryl which had led to the closing down of a plant on account of serious accidents. As a result of his work the plant was re-started and subsequently ran without difficulties.

Nolan managed, however, to maintain his interest in pure organic and biochemical research, and with Professor (now Sir Robert) Robinson he published in the J. Chem. Soc. a research on the synthesis of pæonidin. They confirmed the formula for its constitution which Nolan had suggested some ten years earlier.

In 1925 Nolan succeeded Dr. Joseph Reilly as assistant State Chemist in Dublin, and six years later he became State Chemist. In addition to investigational work upon analytical methods, and the normal routine work of his department, Nolan published in the Proceedings of the Royal Irish Academy work on the pigments of the elderberry. The second of the two pigments was shown to be a new type of anthocyanin, which he named "Sambucicyanin."

In 1932 Nolan followed Hugh Ryan as Professor in University College, Dublin. During the remaining thirteen years of his life he investigated products derived from our native lichens. These researches appear in the Scientific Proceedings of our Society, and are familiar to our members as far as their highly specialized character permits. He was the first to isolate a chlorinated depsidone, gangaleoidin, from *Lecanora gangaleoides*, and worked on its constitution and that of other chlorine-containing lichen substances. In the last few years Nolan extracted from *Lecanora epanora* two nitrogenous substances. In these studies he collaborated with his assistants and pupils, who remain to follow up this interesting line of study.

No 17.

ON A RECENT BOG-FLOW IN MEENACHARVY TOWNLAND, CO. DONEGAL.

D. W. BISHOPP AND G. F. MITCHELL.

[Read JUNE 25 Published Separately OCTOBER 15, 1946.]

THE bursting of a bog in West Donegal, said locally to have occurred on the night of January 30th-31st, 1945, was reported to the Director of Geological Survey, who was asked to inspect the site and to endeavour to ascertain whether further bog movement might be expected. The site was examined in co-operation with Mr. Mitchell of Trinity College on February 16th, 1945, when it appeared improbable that the same flow would extend farther. The margins of the flow were subsequently mapped on May 25th, 1945, when conditions of weather and terrain were somewhat improved. The authors wish to acknowledge the valuable assistance in the field given by Mr. Cunningham of Meenaneary, and the courtesy of the Ministers for Industry and Commerce, and Finance, in sanctioning the publication of this paper and map based on O.S. and Geological 6" Sheet Donegal No 81.

The town of Carrick is situated in a long valley that runs from north to south. The valley stretches about six miles north of the town, and is occupied by the Glen River. About half a mile south of the town the river becomes tidal, and the southern end of the valley is a narrow inlet of the sea about two miles long known as Teelin Bay or Harbour. About three and a half miles above the town the Glen River (here flowing at about 300' O.D.) receives two large tributaries, which drain some low ground to the east. On the west side of the river there is a block of higher ground rising to Meenacharvy Hill (857') on the north and Meenaneary Hill (680') on the south. Between these hills there is a small valley in which are two streams draining east to the Glen River. It was from the north-eastern slope of Meenaneary Hill that the bog-flow moved into this valley and thence to the Glen River. The flow had taken place during a night of heavy rain following a period of severe weather.

The top of Meenaneary Hill is a flattened dome and is surrounded by gently sloping ground which is thickly covered by blanket bog. The surface of the bog shows the typical vegetation rich in *Scirpus* and *Eriophorum*. The angle of slope to the north-east is at first less than 6° but at about 500' there is an abrupt change in the slope which becomes steeper reaching an inclination of about 12°. The

peat extends down to the change in slope but there is none below it where rough grass provides some coarse grazing. Near the stream the slope decreases again and there are a few small arable fields. The valley of the modern stream is cut into this flatter ground north of the stream, its left bank rises steeply. Bog occurs again on the flatter ground along the valley of the Glen River. Here in places it has been drained and its surface brought under cultivation.

The end of the bog at the change in gradient forms a well-drained firm margin, and the flow seemed to have been started by the movement of a sector of this margin down the steeper slope below. Once the firm edge had given way the peat above was left unsupported on one side and began to break away and move downhill in large blocks, much, apparently, as icebergs break away from the front of a glacier where it enters the sea. The break in the margin was about 70 yards wide, and a narrow channel of about this width ran back up the sloping hillside for about half a mile and ended less than two hundred yards north of the summit. A large quantity of peat had moved out through the break, and the channel itself was full of blocks of peat that had come to a standstill before reaching the change in slope. At the upper end the channel widened out to a broader area which was covered with blocks of peat that had not moved any great distance. On the flattest ground at the summit of the hill deep gullies dissected the peat cover into hummocks with abundant *Calluna*; this area had not been affected by the flow.

Just above the limit of disturbance (at a point marked on the accompanying map) the following profile was recorded by means of a peat-auger of the Hiller type :—

- 0—40 ins. Fresh wet fibrous peat.
- 40—65 ins. Highly humified wet peat with remains of fibre.
- 65—67 ins. Yellow-grey material* with vegetable debris.
- 67—105 ins. Highly humified peat with remains of fibre.
- 105—118 ins. Highly humified amorphous peat with some fragments of wood (*Betula*).
- 118—124 ins. Crumbling peat with much wood debris.
- At 124 ins. Auger on stone.

The undisturbed peat seemed to be firm throughout, and there was nothing to suggest that above the limits of obvious disturbance there had been any drainage of liquid material from under the upper fibrous layers of peat. The appearance of the debris suggested that the movement of the flow, at least as far as the change in slope, must have been regular rather than turbulent in manner. When the peat at any point was left unsupported on the down-hill side by the movement of material further down the slope the fibres in the upper layers at first bound it to the peat uphill. A certain distance back from the unsupported edge the force of

* Examined in the laboratory this material yielded no recognisable plant remains, it effervesced with dilute hydrochloric acid. Meenaneary Hill is largely composed of metamorphic rock which near the summit includes some micaceous limestone. The material may represent a former channel on the bog surface along which calcareous water drained away. It is probably quite local in occurrence.

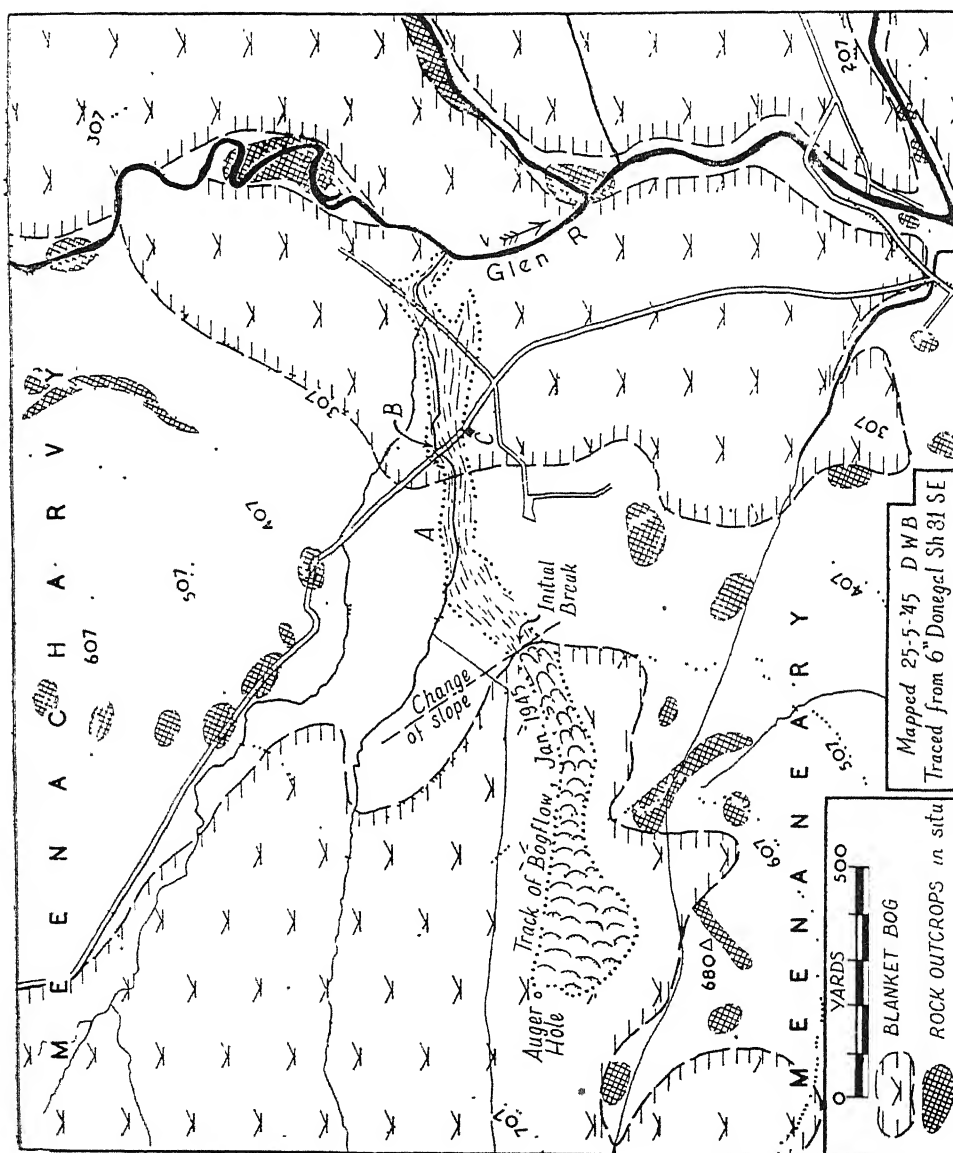
gravity was sufficient to tear the fibres and a block of peat was set free. This then moved down the slope gliding on the lower layers

The lower layers though relatively firm when *in situ* were saturated with water, and it is possible that, when such decayed peat is disturbed, some process analogous to a reversal of phase can take place, and the consistency of the peat may become much more liquid. Below the change in slope the material appeared to have moved as a liquid, but in the channel above the stranded blocks suggested that they had moved forward, shrinking in thickness as the humified lower layers became dissipated, until friction between the fibrous upper layers and the floor of the channel brought the block to a halt.

At one point where a knoll of drier ground abutted against the line of the flow the channel narrowed considerably. When the blocks set free in the wider area above entered this narrow part of the channel the congestion forced some of them laterally out of the channel up on to the undisturbed bog on either side where they became stranded. One large block moving down from above carried on its upper surface a large clamp of turf about fifteen feet long, six feet high, and three feet wide. The clamp must have been carefully built because, though the block which supported it rose at least six feet as it mounted up on to the bog surface beside the narrow part of the channel, the sods in the clamp were not seriously disturbed. Another clamp built on the undisturbed bog surface beside the channel had flowed material heaped up against it, but was not carried away. Some turf cuttings were obliterated by the flow

The outline of the area from which the peat had moved down suggested that there was a depression of similar outline on the surface of the slope beneath the peat, and that a natural drainage basin had been emptied. It was thought accordingly that no further movement was likely. To the west of the bog-flow there was on the hillside a similar long narrow depression, clothed in heather and rushes, which seemed to be the site of an earlier flow down the same valley, and it is quite possible that at some later date a further yielding of the bank of peat at a new point in the change in slope may set more peat moving downhill.

When the material poured through the gap at the change in slope it spread radially before swinging to the left and following the course of a field ditch to the stream below. When this route was established a curved ridge of debris was left on the slope to the right. The grazing slope above and the small fields below were seriously damaged by the flowed material. The debris followed a route which brought it down to enter the stream at right angles to the valley of the latter, so that the flow mounted up the steep northern bank of the stream leaving an arc of debris to indicate the highest level reached (A on map). Its further course was at first confined to the inner valley of the stream, but where the latter passed under a bridge (B on map) it overflowed and damaged the road near the bridge. Though a cottage (C on map) which stood within a few yards of the bridge had been very closely approached by the flow, its inhabitants had not been disturbed. The stream was probably in flood at the time, and in a night of storm and rain no unusually increased volume of sound was noted by them. Below the bridge the



Based on the Ordnance Survey by permission of the Minister for Finance.

flow spread on over the boggy ground beside the Glen River, and here it covered some reclaimed land and a section of road. A considerable amount of debris reached the Glen River, which was also swollen at the time, and stranded blocks of fibrous peat could be seen along the banks of the River for some miles towards Carrick. The flood in the Glen River prevented the flow from building a dam across the valley.

This bog-flow had a very close resemblance to one that occurred in Co. Clare in 1934 (1). There the bog surface was interrupted by escarpments in the underlying rock, and above each escarpment was a firm bank of well-drained peat: at one escarpment the bank gave way, and peat poured through the gap till a narrow channel running back uphill had been opened right up to the watershed. In the account of the flow it was suggested that the breach was due to the heavy rainfall of the previous week increasing the weight of the bog, and so increasing its thrust against the lower bank of peat, till the latter collapsed over the escarpment initiating the flow. Sudden heavy rainfall (1.80" in 3 hours) is also suggested as the cause of a recent bog-flow in Yorkshire (2).

It is possible that a similar cause brought about the Meenachary flow. For some time before the burst the hill slopes had been covered by snow, whose melting was checked by prolonged frost. A sudden thaw accompanied warm air and heavy rain, and the weight of the rain combined with that of the snow may have caused the collapse of the margin of the bog above the change in slope. It is also possible that the severe frost affected the peat in the exposed margin and weakened it. It seemed fairly certain that it was the collapse of the margin that started the flow.

Such a bog-flow suggests that its movement released a tension that had been slowly accumulating, and it seems the most simple solution to attribute the accumulation of tension to a continued growth of the bog. But the dissected state of the peat cover on top of the hill suggests that here erosion rather than accumulation is going on. On many of the tops of the Wicklow Mountains serious erosion of the peat cover is taking place. Yet two bog flows have occurred in recent years on the flanks of these mountains. (3, 4).

The records of the comparatively infrequent bog-flows that have taken place during the last 200 years describe in each case a simple set of events. It is obvious that a bog must burst or slide when its gravity-load stresses exceed the restraining forces of coherence, internal friction, surface tension, or whatever they may be. We are, however, far from being able to predict when a bog is approaching its critical or danger point, and how it may be controlled. At least four factors may be involved.

- (1) Excessive precipitation, which almost always seems to precede a burst, may be the trigger-action which releases a dangerous bog. The excess loading however is relatively small. A two-inch rainfall is equivalent

to 200 tons per acre ;* but this only brings about an increase of 2" in the head of water on a bog over 10 feet thick.

- (2) The sudden addition of a small percentage of water to what may amount to a colloid suspension may lead to a sudden disastrous increase of fluidity.
- (3) Disturbance of the bog, *e.g.*, by tremors induced by wind-pressure, may have exactly the same effect as (2).
- (4) If there are any gases being generated in, or dissolved in, a bog, a sudden fall in barometric pressure may release these causing increase of fluidity. The release of methane in coal mines following a barometric 'low' is well-known.

It appears not unreasonable to enquire what might be the effect of treating a bog *in situ* with salt water or other electrolytes.

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- (3) MITCHELL—Scient. Proc. R.D.S., 22 (N.S.), No. 4, 1938.
- (4) DELAP and MITCHELL—Scient. Proc. R.D.S., 22 (N.S.), No. 18, 1939.

* Opportunity is taken here to correct an error in an earlier paper (1). There on p. 249 it is stated that 2 ins of rain is the equivalent of 65 tons per acre instead of 200 tons per acre.

No. 18

ON A RECENT ADDITION TO THE COLLECTION OF IRISH METEORITES
IN THE NATIONAL MUSEUM DUBLIN.

By

H. J. SEYMOUR, B.Sc., B.A., M.R.I.A.
(Professor of Geology, University College, Dublin)

[Read NOVEMBER 26, 1946. Published separately May 15, 1947]

(PLATE 12)

SINCE the year 1795 falls of meteorites represented by museum specimens have numbered only sixteen in these Western Islands. Seven of the falls occurred in England, five in Ireland, three in Scotland, and one, the latest in date (April 14th 1931), at Pontlyfni, Carnarvonshire, in Wales. A phenomenon of this nature is sufficiently uncommon to attract considerable attention at the time of its occurrence, but not infrequently some of the fragments recovered are lost or mislaid subsequently, especially if they pass into private hands.

Such fortunately was not the ultimate fate of the present meteorite, the largest individual fragment yet known to have fallen in Ireland, or indeed in Great Britain, viz. one of the component parts of the 'Limerick' meteoritic shower of September 10th, 1813. All the stones of this particular fall were dispersed over an area lying between Faha House (north of Patrickswell) and Adare, some three miles distant to the south-west. There were recovered ultimately three large and some six or seven smaller pieces, the weights of the former being recorded as about 65, 24, and 17 lbs. respectively. It was known that the 17 lb. specimen is in the Oxford University museum, and that the smaller stones are in various British, Irish, and Continental museums, but the whereabouts of the two larger ones was not hitherto known in scientific circles. This is still true of the 24 lb. specimen, but the largest one, commonly referred to as the '*Brasky*' mass, has again come to light and is the subject of the present paper. Though it fell in 1813, no detailed description of it has hitherto been published, only the approximate weight and shape being noted in the more or less contemporaneous accounts of the incident.

Its re-discovery is due to Mr. J. A. Morrison of the Irish Land Commission, who late in November 1945 in the course of a routine inspection of a recently allocated holding near Rathkeal, Co. Limerick, noticed in the house of a small farmer, Mr. John Collins of Hollypark, Kilkornan, Co. Limerick, a large stony mass which he

recognised as of extra-terrestrial origin. He advised the owner to have it examined at the National Museum, at the same time notifying Dr. O'Connor, the Acting Director, of its location. On communicating with Mr Collins, the latter readily consented to forward it to the Museum, where I made a superficial examination, and pointed out the desirability of its purchase for our mineral gallery. This was ultimately arranged to the satisfaction of the parties concerned, and this fine meteorite became museum property. Before the negotiations had been concluded, Mr. Collins gave permission for the removal of a small chip for micro and chemical examination, and I had a section prepared which was later seen and compared with sections of the same fall in the British Museum, by Dr Hey. In a letter to me he states :—

“ We have compared it with our specimens and we are satisfied that there is no visible difference and that the mass can certainly be accepted as authentic. It is a veined grey chondrite agreeing in all particulars with the description of the Limerick mass quoted in Prior's catalogue (3), and the fragments and sections agree perfectly.”

There is therefore little doubt but that our recent find is an integral portion of the meteoritic shower of 1813.

The tradition in the Collins family is that the ‘thunderbolt’ remained in the possession of the Tuthills of Faha House, whose then owner recovered it from his lands following its observed fall. Later it passed into the custody of the Taylors of Hollypark (near Askeaton) on intermarriage with the Tuthills. When their estate was purchased some seven or eight years ago from its owner, Miss C. E. Taylor, an auction was held at which the meteorite was acquired by Mr. Collins. According to the latter it was not put up separately but included in a miscellaneous lot and

“ no one took any particular interest in it and I thought it of no earthly value, other than its having a sentimental one for me, since our family had worked for generations for the Tuthill and Taylor families, and my father (now aged 85) was also glad to have the stone as a souvenir.”

Mr. John Collins stated further that it was his own grandfather who over seventy years ago, actually brought this meteorite, with other articles of furniture, etc. from Faha House to the home of the Taylors at Hollypark. It would appear therefore that this particular specimen has been what may be described as “under observation” by some member of the Collins family for much more than eighty years, if not indeed for the whole period since its finding in 1813.

Discrepancies in the weights recorded for meteorites are of common occurrence, so that the fact that our specimen weighs some five and three quarter pounds less than the reputed weight of the Brasky mass, does not necessarily imply that it is a separate entity. This alternative would be most unlikely, since such a large mass could not possibly be preserved and yet remain unknown to the local magnates in a typical rural area. It is much more probable that the originally recorded weight was incorrect by reason of the methods or apparatus used.

The first account of the Limerick episode appeared in the local newspaper, the *Limerick Chronicle* of Sept. 11th 1813 as follows—

"Yesterday morning about nine o'clock, there was the most dreadful thunder heard in the direction of from Patrickswell towards Adare and Askeaton, in this county; the peals were very violent, continued for some time and were accompanied with some awful appearances, large fragments of atmospheric stones, and other circumstances, which indicated some very serious concussion to have taken place."

The earliest account, in a scientific publication, appeared in the *Philosophical Magazine* of 1818 (2), five years after the event, in the form of a communication to its Editor from William Higgins, Professor of Chemistry to the Dublin Society, now the Royal Dublin Society. It was accompanied by a copy of a letter forwarded to Higgins by one Samuel Maxwell of Imerick, and containing information about the fall of 1813 supplied by Mr. George Tuthill, near whose home (Faha House) the meteoritic shower occurred. This is the communication commonly referred to as Maxwell's letter though in fact it is Tuthill's.

For our purpose the relevant data contained therein are as follows:—

"One (meteorite) fell on the lands of Scagh in the neighbourhood of Patrickswell and was immediately dug up, being warm and weighing 17 lbs. about. Six or seven similar but smaller stones descended between the lands of Scagh and the village of Adare.

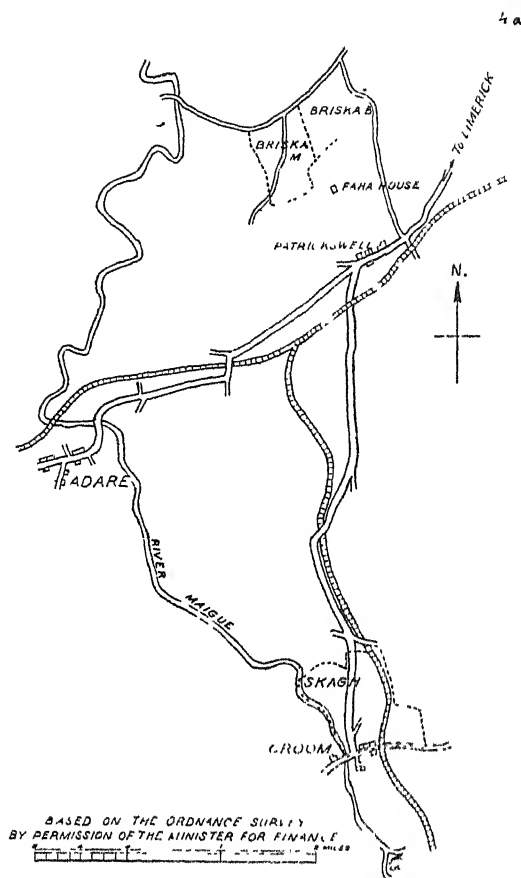
One more very large mass came to ground on the lands of Brasky, buried itself to a depth of two feet, and was not dug up for two days, it was fractured in places and weighed about sixty-five pounds. Its shape was rather round but irregular.

At the same time there fell on the lands of Faha another stone unfractured, of irregular shape and weighing twenty-four pounds, which is now in my (Tuthill's) possession.

It is three miles from the lands of Brasky where the large stone fell, to the village of Adare, where the small stones were picked up, and all the others fell intermediately."

Another account of the same event appears in a brief footnote on page 430 of Linehan's '*Limerick and its Antiquities*' published in the year 1866, and therein it is mentioned that one of the stones is in the possession of Mr. Tuthill, and weighs four stones weight. It may be assumed from the foregoing that the two largest fragments at least were once in the possession of Mr. Tuthill, and that the special reference to the 24 lb. specimen in his own account above, was due to its being unfractured, thus differing from the 65 lb. mass.

An examination of the Ordnance six-inch maps show that the Faha demesne on which the 24 lb. fragment fell, is bounded on the north and north-west by the two townlands of Briska More and Briska Beg. Locally Briska is pronounced as if spelled Brisky, and this is clearly the 'Brasky' area of Maxwell's letter. The precise situation of the 'lands of Scagh' is not readily determinable. There is a townland of Skagh some five miles to the south near the village of Croom, but this cannot be regarded as being 'in the neighbourhood of Patrickswell' nor does it occupy an intermediate position between Brasky and Adare, stated to have been the terminal points of the track of the Limerick meteoritic shower of 1813.



The relative positions of the places named here are shown on the accompanying map based on the 1" Ordnance sheets, 143 and 153. From all the evidence available it seems probable that locally in the past, the term 'lands of Scagh' indicated the general area of the two Briska townlands and further, that all the larger fragments fell in the vicinity of Faha House and demesne. In this connection it may be noted that the account in Maxwell's communication suggested a direction of travel for the stones from east to west. Generally since smaller objects encounter more resistance relatively to their mass than do the larger ones in any meteoritic shower, the former should reach the ground at nearer points and at a steeper angles, whereas the latter owing to their greater momentum will normally carry further in the direction of travel. The known distribution of the fragments of this shower would therefore suggest a motion from west to east, or actually south-west to north-east.

The general form is well shown in the accompanying photographs (Plate 12), which also indicate the essentially irregular characteristics of a typical rock fragment. Roughly speaking its shape is that of an irregular three-faced truncated double

pyramid, the overall dimensions being about 32 x 26 x 21 cm, or say $12\frac{1}{2}$ x 10 x $8\frac{1}{2}$ inches. About two-thirds of the specimen lies above the level of its greatest width, and one-third below (Fig 1). There are numerous faces of varying sizes, some slightly concave and others convex, and mostly glazed all over with a thin brownish black skin of fused-rock material. The faces vary in type, the larger number being deeply 'thumb-marked' and slightly concave (Figs. 1 and 2). About one-third of the total surface area consists of relatively smooth and slightly convex faces, probably of later origin, but with a well fused crust on which incipient 'thumb, marks' are rather sparsely distributed (Fig 3). Another type of face constituting the larger proportion of the residual surface, shows only partial or incipient glazing in the form of flow ridges or flashings of fused material, in some places more conspicuously developed than in others. (Fig 2). These illustrate a stage preliminary to the formation of an all over surface crust, and in the present case clearly belong to a late period of the meteorite's travels through our atmosphere. At the base and elsewhere small fracture surfaces, without any obvious glaze, occur, and are referable to some form of impact, and to the same cause may be due the two prominent fissure planes which show up on the base. (Fig. 4)

In its present state the meteorite weighs 27,060 grams, or say fifty-nine pounds, four and one quarter ounces. The weight of the chip removed prior to this weighing was 42 grams, and from this a thin section was made and material for chemical analysis supplied. Two small pieces remain weighing together a little over 19 grams.

The density as determined from this chip was found to be 3.84, that of a fragment from Adare in the Trinity College, Dublin, collection was 3.64. A specimen analysed by Apjohn (1) in 1874, is recorded as having a density of 3.94, from which it would appear that the distribution of the nickel-iron content in the meteorite is irregular.

This meteorite belongs to the group known as aerolites, which consist essentially of stony matter, of a basic mineral nature, with a low nickel-iron content. Usually their constituents occur as 'chondrules' or rounded aggregates of the minerals present, in which case the aerolites are classed as 'chondrites.' The latter are again sub-divided according to the percentage of nickel in the nickel-iron present, notice being also taken of structure, colour and mineral composition. Our meteorite is accordingly classed as a *veined grey bronzite-chondrite*.

Under the microscope the section reveals the presence of numerous chondrules, varying in diameters between 0.3 and 1.2 mm., and fairly evenly dispersed in a crystalline matrix, in which there is a good deal of nickel-iron and scarce troilite. (Fig. 5). The small and well rounded chondrules consist mostly of olivine crystals, while the larger and less well rounded ones are composed of aggregates of elongated crystals of bronzite, associated with a small amount of olivine and dark interstitial matter. In one or two of the smaller chondrules and elsewhere in the section some colourless material suggests the presence of feldspars having a lower refractive index than Canada balsam.

An analysis of one of the Adare fragments of the Limerick fall has been published by Prior (5.) and from it he deduces the mineral composition as follows:—

| | | |
|-------------|-----------------|-------|
| | | % |
| Felspar | (Na, K, Ca.) | 7.53 |
| Chromite | | 0.87 |
| Apatite | (Merrillite ?) | 0.63 |
| Bronzite | (MgO/FeO = 5) | 33.83 |
| Olvine | (MgO/FeO = 4) | 32.64 |
| Nickel-iron | (Fe/Ni = 11) | 18.46 |
| Troilite | (FeS) | 5.60 |
| | | 99.56 |

A partial analysis of the magnetically attracted portion only, of the powder got by grinding down a fragment of the meteorite, was also made according to a method which the same author devised and published in 1919 (4). A portion of the recent find was analysed for comparison, by the same method in our State Laboratory, and both results are recorded below.

| | | Prior | State Laboratory |
|---------------------------|-----|----------------|----------------------|
| | | % | % |
| Insoluble silicate (i) | ... | 22.35 | 9.10 |
| Soluble silicate | ... | 16.64 | 4.39 |
| FeS (t) | ... | 1.93 | 0.60 |
| Ni (n) | ... | 4.93 | 7.23 |
| Fe (+ Co) by difference | .. | (54.15) | (78.68) |
| P | ... | — | traces not estimated |
| | | 100.00 | 100.00 |
| Weight of Attracted | ... | 2.7384 g. | 2.636 g. |
| „ „ Unattracted | ... | 6.1524 „ | 10.493 „ |
| Percentage of Nickel-iron | | 18 | 17.26 |
| Ratio of Fe (+ Co) to Ni | | 11 | 10.88 |
| Group. | | C ₂ | C ₂ |

It will be observed that there are concordant results in both analyses in respect of the nickel-iron content and also for the iron-nickel ratio. A separate analysis to determine the Fe + Co content of the attracted portion of our meteorite gave 78.50%, which corresponds with that got by 'difference'. The variation in the silicate content is due to the fact that Prior's aim was primarily to obtain a metal-free silicate residue. In our case the endeavour was essentially to obtain a metallic residue free from silicate. The method of separation by magnets in any event depends on so many varying factors that concordant results cannot be expected in all cases.

The following is a list of the British and Irish meteoritic falls, represented by museum specimens, which have been recorded since 1795. The arrangement is geographical, and only the weight and present location of the largest and heaviest fragment now extant are recorded below for each individual fall.

| <i>England.</i> | <i>Date of fall.</i> | <i>Wt. in grams.</i> | <i>Repository.</i> |
|-------------------------|----------------------|--------------------------|--------------------|
| Wold Cottage, Yorkshire | ... 13/12/1795 | 20,638 | British Museum |
| Launton, Oxfordshire | ... 15/2/1830 | 998 | „ „ |

| | | | |
|--------------------------------|------------|--------|-----------------|
| Aldsworth, Gloucestershire . . | 4/8/1835 | 520 | „ „ |
| Rowton, Shropshire ... | 20/4/1876 | 3,109 | „ „ |
| Middlesborough, Yorkshire . | 14/3/1881 | 1,546 | York Museum. |
| Appley Bridge, Wigan, Lancs. | 13/10/1914 | 10,900 | British Museum. |
| Ashdon, Essex ... | 9/3/1923 | 1,270 | „ „ |

Ireland.

| | | | |
|--------------------------|-----------|--------|-----------------------------|
| Moorsfort, Tipperary ... | —/8/1810 | 1,208 | National Museum, Dublin. |
| Adare, Limerick ... | 10/9/1813 | 27,060 | „ |
| Killeter, Tyrone . | 29/4/1844 | 90 | British Museum. |
| Dundrum, Tipperary .. | 12/8/1865 | 1,638 | Trinity College, Dublin. |
| Crumlin, Antrim . . | 13/9/1902 | 3,821 | British Museum |

Scotland

| | | | |
|----------------------------|-----------|-------|--------------------------------------|
| High Possil, Glasgow ... | 5/4/1804 | 91 | British Museum. |
| Perth, Perthshire ... | 17/5/1830 | 1½ | „ „ |
| Strathmore, Perthshire ... | 3/12/1917 | 9,911 | Royal Scottish Museum, Edinburgh. |

Wales.

| | | | |
|------------------------------|-----------|-----|----------|
| Pontlyfni, Carnarvonshire .. | 14/4/1931 | 142 | Private. |
|------------------------------|-----------|-----|----------|

In conclusion the writer desires to express his personal indebtedness to Dr. Hey of the British Museum and Dr. Spiller of the Oxford University Museum for their kind assistance and interest. He wishes also to thank Dr. O'Connor for giving him the privilege of describing this historic meteorite, and for his ready co-operation in the negotiations leading up to its purchase. Thanks are specially due to Mr. J. A. Morrison of the Irish Land Commission, who is primarily responsible for the re-discovery of the Meteorite, and also to Mr. J. A. Collins, who, if his means had permitted, would have been willing to present it to the National Museum once its scientific interest had been brought to his notice. Messrs. Avery of Middle Abbey Street, Dublin, were good enough to furnish facilities for the accurate weighing of this heavy specimen, and Mr. K. O'Kelly and Mr. D. Ryan prepared the map reproduced here.

Models are being prepared in the workshops of the Museum for ultimate distribution to selected institutions and overseas museums as well as to Mr. John Collins its previous owner.

The photographs (1, 2 and 3) on Plate 12 are so arranged that they represent successive faces of the specimen on rotation in an anti-clockwise direction, about an axis coincident with the direction of maximum length of the meteoritic.

EXPLANATION OF PLATE 12.

- Fig. 1. The face here consists of two slightly concave surfaces separated by a low sinuous ridge, each section showing characteristic 'thumb-marks.' The dominant bi-pyramidal form is noticeable. Maximum dimensions 32 x 25 cm.
- Fig. 2. A smaller face also 'thumb-marked' and nearly flat. On the left are fracture surfaces almost entirely in the third stage of development but some are of still later origin. One of the former is conspicuous on the N.W. margin, showing darker in colour, with distinct parallel fused or flow ridges running N.E. and S.W. Maximum dimensions 32 x 22 cm. (excluding part of face 1 on right below)
- Fig. 3. The widest face of the meteorite, well glazed, rather smooth, and slightly convex, showing comparatively few ill-developed 'thumb-marks.' One of the two fracture planes can be traced on the right. A small thumb-marked face occurs below. This is the only aspect of the meteorite that approximates to Tuthill's description as "round but irregular." Maximum dimensions 32 x 27 cm.
- Fig. 4. Base of meteorite (the face at right angles to direction of maximum elongation which is vertical in Figs 1, 2 and 3) showing on the more recent fracture surfaces the character of the grey crystalline matrix. Most of these surfaces show a flash of incipient glazing. One completely fused face, like that predominating in Fig. 3, occurs on the right, while above, portions of the thumb-marked (1 and 2) faces are seen. The two intersecting fracture planes may also be traced in the photograph. Maximum dimensions 28 x 22 cm.
- Fig. 5. Micro section (x 10) showing chondrules. Most of the dark material is nickel-iron. The dark more-or-less rectangular crystal below the large round chondrule is troilite, and portion of a typical olivine chondrule occurs near it. The oval-shaped chondrule at top of section consist mainly of bronzite crystals.

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- (5) " " **19**. p. 169. 1921.



FIG. 1

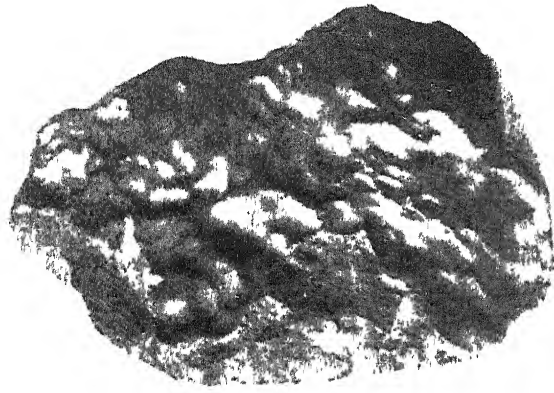


FIG. 2

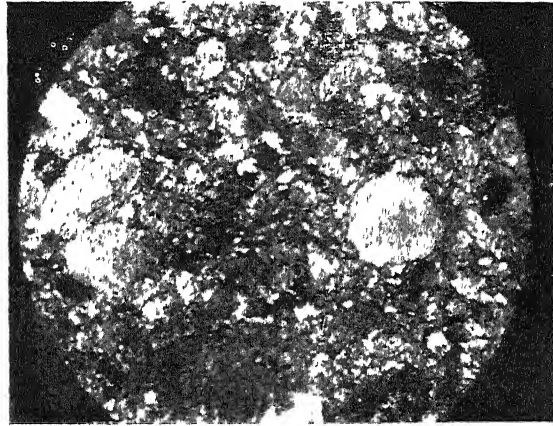
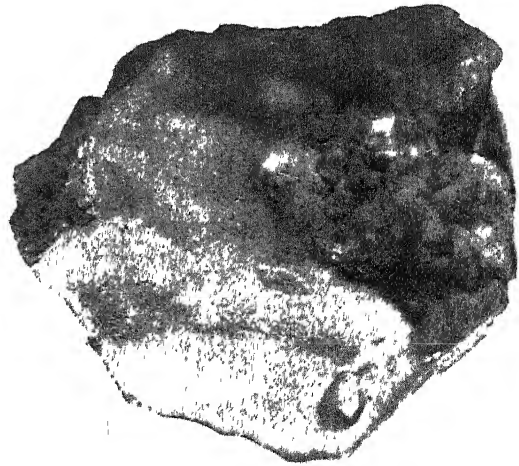


FIG. 5



No. 19.

IRISH SALMON, 1945.

By

ARTHUR E. J. WENT,

(Department of Agriculture, Fisheries Branch, Dublin)

[Read NOVEMBER 26, 1946. Published separately May 17, 1947.]

IN a preliminary investigation made in the year 1944 (Went, 1945) the proportion of previously spawned fish in a number of Irish rivers was determined. On that occasion the material consisted of a single scale taken from just below the dorsal fin from about 25,000 salmon captured in some twenty five Irish rivers. Such material was satisfactory for the purpose for which it was intended, but in 1945 it was decided to modify the method of collection, and the collectors were asked to scrape off a few scales from the region just below the dorsal fin in the case of as many fish as possible, and to preserve the scales from each individual fish in separate envelopes, specially provided for the purpose. The scales from each month were to be kept separate. In this way it was hoped to obtain some information additional to that available in the previous year.

Over 8,300 sets of scales were obtained from 25 localities, eight rivers furnishing inadequate material consisting of less than 100 sets of scales. A list of the rivers investigated, together with the names of the persons collecting the material, has been given in Table 1 (see Appendix for all tables). The writer wishes to take this opportunity of thanking the collectors, without whose collaboration this investigation would have been impossible.

METHODS.

A typical scale selected from each sample was cleaned in dilute sodium peroxide solution, and was mounted with the aid of dilute gelatine solution on a microscope slide. Subsequently the scales were examined under the microscope, and the details of the life history of the individual fish ascertained.

SMOLT AGES.

The fish were divided into four smolt classes, namely the one-, two-, three-, and four-year smolts (see Table 2). In every river the bulk of the fish belonged to the two-year smolt class, and, save in the case of the Waterville, Sligo, and Erne

Rivers, the one-year smolts were more important than the three-year smolts. Only two four-year smolts were observed in the present material, showing clearly that this smolt class is unimportant in Irish salmon.

One might now ask how the present results compare with the previous investigations on some of the rivers investigated in 1945. In Table 3 the present results were compared with the results obtained in previous investigations. In general, the order of the values obtained is the same, the only noticeable exception being that of the Corrib in which in 1945 the proportion of three-year smolts showed some diminution. Even in this case the results are such as might be expected in a normal river from year to year.

SMOLT TYPES.

In previous investigations on Irish salmon (Went, 1938-1946) it was mentioned that some fish showed marked growth in fresh water in the spring immediately prior to migration as a smolt, whereas other fish showed no such growth. Those fish showing marked growth were, for convenience, designated type B smolts, and those in which growth was limited to two new growth elements or less were designated type A smolts. In the present material the division into the two types in the various smolt classes has been made and the results are given in Table 4.

The two-year type B smolts formed the bulk of the fish in every river investigated in 1945. A similar condition was also observed in most of the previous investigations on Irish salmon, the only notable exception being the Ballisodare River (see Table 5).

AGE GROUPS.

The distribution of the different age groups (as percentages of the total catch) in the various rivers investigated have been indicated in Table 6. Local and other inquiries have shown, however, that in 1945, apart from the poor runs of fish generally, grilse were scarce. This was particularly noticeable in the case of the Rivers Boyne, Waterville and Lee. In addition the large spring fish, have for some years, decreased considerably in numbers in many rivers throughout Ireland.

Whilst the present results do not afford an exact indication of the proportion of the various age groups, owing to the unavoidable fluctuations in the proportion of fish sampled throughout the season, the results do give a fairly clear idea of the runs of fish into the rivers in question. It seems probable also that the figures given in the last line of Table 6 represent, to some degree, the conditions prevailing in Ireland generally in 1945. Certainly the grilse (1 + winters) and the small spring fish (2 winters) are the most important age groups, followed by the small summer fish (2 + winters) and either the large spring fish (3 winters) or the previous spawners (with SMs). The large summer fish (3 + winters) and the very large spring fish (4 winters) are unimportant in the stocks of salmon in the country as a whole. They are, in fact, to be found in very few rivers throughout the country.

A comparison of the present results with those of previous investigations as regards the distribution of the various age groups is given in Table 7. The only comment necessary in this connection is that the proportions of grilse (1 + winters)

in the 1945 runs were less than in previous years, additional proof, if such was necessary, of the previous statement that these fish were scarce in 1945 in most Irish rivers. A later section will be devoted to consideration of the previously spawned fish.

DISTRIBUTION OF THE YEAR CLASSES (BROOD OF A PARTICULAR YEAR).

In Table 8 the distribution of the fish in the various rivers into the appropriate year classes has been given. No general condition can be followed in the various rivers except that the bulk of the fish in all the rivers were spread over not more than two year classes. There was, in fact, almost a complete gradation from the state existing in the Corrib and Moy, where the two most important year classes were approximately of the same strength, to the Kerry Blackwater, where nearly 90% of the fish were derived from the one year class. Taking the country as a whole four-year-old fish (i.e. the 1941 year class) were more important than three-year-old fish (i.e. the 1942 year class).

A comparison of the present results with those of previous investigations has been made, Table 9. The more important differences exhibited in this respect can be attributed in the main to the reduced runs and catches of grilse in 1945.

THE PREVIOUS SPAWNERS

In a previous paper (Went, 1945) dealing with Irish previously spawned salmon it was clear that there was a great variation in the proportion of previously spawned fish in the different rivers. In 1944 the variation was from 0.6% in the River Liffey to 9.5% in the River Erne. During the present investigation an even greater variation was found (see Table below) namely from 0.6% in the River Boyne to 15.4% in the River Erne.

| RIVER | Percentage of previous spawners in 1945 | Percentage of previous spawners in 1944 |
|-------------------------------|---|---|
| Boyne | 0.6 | 1.2 |
| Lee | 1.0 | 1.2 |
| Liffey | 2.3 | 0.6 |
| Ballisodare | 2.9 | 2.4 |
| Cork Blackwater | 3.2 | 2.5 |
| Feale | 3.9 | 1.8 |
| Kerry Blackwater | 4.0 | 5.1 |
| Sligo | 4.8 | 4.5 |
| Laune | 5.4 | 3.3 |
| Shannon | 5.7 | 4.1 |
| Bandon | 5.9 | 8.3 |
| Waterville | 7.3 | 5.8 |
| Moy | 8.2 | 4.3 |
| Corrib | 11.2 | 7.5 |
| Bundrowes | 11.4 | 7.3 |
| Erne | 15.4 | 9.5 |
| Total for all rivers combined | 5.4 | 4.3 |

In general, the proportion of previously spawned fish was higher in 1945 than in 1944. It is also noteworthy that in rivers in which the proportion of previously spawned fish was very low in 1944 (Rivers Liffey, Lee, and Boyne) it was also low in 1945. Similarly where in 1944 the proportion of previous spawners was very high (Rivers Bundrowes, Corrib, and Erne) it was also very high in 1945. It is unfortunate that sufficient material was not available from the Glen, which in 1944 showed a high proportion of previous spawners. With the exception of the Bandon River, all the rivers having more than the average proportion of previous spawners had on the river system one or more large lakes through which salmon had to pass to reach the sea. On the other hand in cases where the proportion was low there were no large lakes frequented by salmon on the river. The Ballisodare River has a sizeable lake (Lough Arrow) on one of its tributaries, but salmon do not run into the lake. The very high proportions for the Moy, Corrib, Bundrowes, and Erne in 1945 can be attributed partly to the exceedingly poor runs of grilse. With the exception of the Bundrowes, the other three rivers rely for the most part on grilse, which were very scarce in 1945 throughout most rivers in Ireland.

In all, the scales of 460 fish having spawning marks thereon examined, 428 (93%) had one spawning mark, 30 (6.5%) had two spawning marks, and 2 (0.5%) had three spawning marks. The two fish from the River Erne having three spawning marks were the first of this type to be observed in investigations on salmon from Irish rivers. Previous spawners can be classified on (a) the length of sea life before the first spawning, and (b) the absence habit or the period spent feeding between what would have been, but for the intervention of capture, two successive spawnings. In Table 10 the fish have been so classified. Excluding those fish which one could identify merely as having been previous spawners, there were 425 fish the "absence habit" of which could be determined. Of these 211 (49.7%) exhibited the short absence habit (less than a full year), 197 (46.3%) exhibited the long absence habit (a full year), 16 (3.8%) exhibited the very long absence habit (more than a full year) and one appeared to have been two years feeding in the sea between its descent as a kelt and reappearance as a clean fish. As regards the age at first spawning, there was a tendency for the proportion of fish to decrease as the age groups are ascended. For example, fish which first spawned as grilse comprised 61.1% of all previous spawners, those spawning first after two years sea life 34.6% and so on. It seems probable that the smaller fish tend to survive to a much greater extent after spawning than the larger fish.

RÉSUMÉ.

1. Collections of scales were made in a number of Irish rivers during the salmon season of 1945.
2. Scales representing 8,300 fish from twenty-five Irish rivers were mounted and examined microscopically.
3. Two-year-old smolts were most important in all rivers examined (Tables 2 and 3), and the one-year-olds were more important than the three-year-olds in all rivers except the Waterville, Sligo, and Erne Rivers.
4. Two-year type B smolts formed the most important smolt type in every river examined (Tables 4 and 5).

5 The age groups in which the fish have spent less than three full years feeding in the sea together formed over three-quarters of the total in every river (Tables 6 and 7) Gulse were scarcer in 1945 than usual, as were also the large spring fish

6. In most rivers two year-classes (i.e. the brood of two years) accounted for the bulk of the fish There was, however, a gradation from the condition found in the Kerry Blackwater where one year-class accounted for 88.5% of the fish to the conditions found in the Corrib and Moy where the two most important year classes were almost of equal strength. (Tables 8 and 9)

7. The proportion of previous spawners ranged from 0.6% (Boyne) to 15.4% (Erne), the average for all rivers being 5.4% which is somewhat higher than in 1944. The order of the rivers arranged in 1945 (see Table page 107) was similar to that for 1944. Of the fish having spawning marks on their scales 93% had one spawning mark, 6.5% two spawning marks, and 0.5% three spawning marks. The absence habit and age at first spawning was indicated (Table 10)

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APPENDIX.

Table I. Sources of material and collectors. The rivers are arranged clockwise from Dublin.

| RIVER | NAME OF COLLECTOR |
|--------------------|---------------------------------|
| Liffey | Mrs. French Davis |
| Blackwater (Cork) | Mr. G. Fitzgerald |
| Lee | Mr. J. Scanlon |
| Bandon | Mr. W. J. Seymour |
| Ilen | Mr. R. Beamish |
| Sheen | Mr. W. Johnstone |
| Blackwater (Kerry) | Colonel E. Hood |
| Waterville | Mr. J. Butler |
| Laune | Mr. A. de C. Dodd |
| Feale | Mr. G. Gleasure |
| Shannon | Mr. L. Forde |
| Corrib | Lt. Col. E. G. K. Cross, D.S.O. |
| Newport | Mr. M. Kelly |
| Owenduff | Mr. P. J. Lenehan |
| Owenmore | |
| Carrowmore Lake | |
| Moy | Mr. W. P. Stevens |
| Ballisodare | Mr. F. F. Scott |
| Sligo | Mr. W. T. Middleton |
| Bundrowes | Mr. J. Cassidy |
| Erne | Mr. T. Mac D. Swan |
| Owenea | Mr. F. A. Watson |
| Lennon | Sir J. Stewart |
| Crana | Miss H. Moore |
| Boyne | { Capt. R. Law |
| | { Mr. P. J. Tiernan |

Table 2. Distribution of the various smolt ages as percentages of total number of fish examined (maiden fish only)

| RIVER | SMOLT AGE | | | |
|---------------------|-----------|---------|---------|---------|
| | 1 year | 2 years | 3 years | 4 years |
| Liffey | 15.2 | 80.5 | 4.3 | — |
| Blackwater (Cork) | 14.7 | 83.7 | 1.6 | — |
| Lee | 17.8 | 81.2 | 1.0 | — |
| Bandon | 18.9 | 76.5 | 1.0 | — |
| Blackwater (Kerry) | 2.7 | 95.4 | 1.9 | — |
| Waterville | 3.2 | 90.3 | 6.5 | — |
| Laune | 5.4 | 92.4 | 2.2 | — |
| Feale | 2.6 | 96.8 | 0.6 | — |
| Shannon* | 12.8 | 81.0 | 6.2 | — |
| Corrib | 17.4 | 76.1 | 6.3 | 0.2 |
| Moy | 15.4 | 80.0 | 4.6 | — |
| Ballisodare | 14.4 | 82.4 | 3.2 | — |
| Sligo | 5.5 | 87.3 | 7.2 | — |
| Bundrowes | 10.7 | 84.9 | 4.4 | — |
| Erne | 4.8 | 88.6 | 6.6 | — |
| Boyne | 19.3 | 76.6 | 4.0 | 0.1 |
| All rivers combined | 11.9 | 84.1 | 4.0 | + |

* Weighted figures from Went (unpublished data)

+ = trace (less than 0.05%)

Table 3. Comparison of present results with those of previous investigations as regards the distribution of the various smolt ages (As percentages).

| River | Year or years investigated | Reference | Smolt Ages | | | |
|------------------|----------------------------|-----------------------|------------|---------|---------|---------|
| | | | 1 year | 2 years | 3 years | 4 years |
| Liffey | { 1933-35 | Frost and Went, 1940 | 12.2 | 85.4 | 2.4 | — |
| | { 1940-41 | Went, 1946 | 16.6 | 80.5 | 2.9 | — |
| | { 1945 | Present results | 15.2 | 80.5 | 4.3 | — |
| Kerry Blackwater | { 1943 | Unpublished data | 1.3 | 97.4 | 1.3 | — |
| | { 1945 | Present results | 2.7 | 95.4 | 1.9 | — |
| Waterville ... | { 1941 | Went and Barker, 1943 | 5.1 | 86.0 | 8.9 | — |
| | { 1945 | Present results | 3.2 | 90.3 | 6.5 | — |
| Shannon* ... | { 1941 | Went, 1943 | 11.5 | 83.3 | 5.0 | 0.2 |
| | { 1944 | Unpublished data | 8.2 | 78.9 | 12.9 | — |
| | { 1945 | Present results | 12.8 | 81.0 | 6.2 | — |
| Corrib ... | { 1924-26 | Went, 1943 | 11.8 | 75.2 | 12.5 | 0.5 |
| | { 1945 | Present results | 17.4 | 76.1 | 6.3 | 0.2 |
| Ballisodare .. | { 1938 | Went, 1941 | 22.9 | 75.2 | 1.9 | — |
| | { 1939 | Went, 1941 | 18.8 | 80.3 | 0.9 | — |
| | { 1945 | Present results | 14.4 | 82.4 | 3.2 | — |
| Erne .. | { 1928 | Went, 1942 | 4.8 | 92.0 | 3.2 | — |
| | { 1945 | Present results | 4.8 | 88.6 | 6.6 | — |

* Results given only relate to this river since the advent of the hydro-electric scheme

Table 4. Percentage of each smolt type in each smolt class in maiden fish only in the different rivers.

| Smolt Age | Type A | | | | | | | | | | Type B | | | | | | | | | |
|--------------------|---------------------|--------|------|------------|------|--------|-------|------------|------------|-------|--------|---------|--------|------|-------------|-------|-----------|------|-------|--|
| | All rivers combined | Liffey | Cork | Blackwater | Lee | Bandon | Kerry | Blackwater | Waterville | Laune | Feale | Shannon | Corrib | Moy | Ballisodare | Sligo | Bundrowes | Erne | Boyne | |
| 2 | 13.8 | 20.3 | 20.5 | 19.8 | 17.1 | 11.7 | 13.8 | 8.2 | 12.9 | 5.8 | 6.8 | 17.7 | 10.2 | 10.2 | 14.8 | 6.2 | 12.6 | 5.0 | 24.3 | |
| 3 | 2.3 | 2.3 | — | 1.0 | 1.0 | 0.5 | 4.0 | 1.3 | 0.6 | 2.1 | 3.5 | 3.9 | 2.2 | — | — | 4.2 | 2.2 | 2.1 | 3.2 | |
| 4 | + | — | — | — | — | — | — | — | — | — | — | — | 0.2 | — | — | — | — | — | 0.1 | |
| Total | 16.1 | 22.6 | 20.5 | 20.8 | 18.1 | 12.2 | 17.8 | 9.5 | 13.5 | 7.0 | 10.5 | 21.6 | 18.4 | 10.4 | 14.8 | 10.4 | 14.8 | 7.7 | 27.6 | |
| 1 | 11.9 | 15.2 | 14.7 | 17.8 | 18.9 | 2.7 | 3.2 | 5.3 | 2.6 | 12.8 | 17.4 | 15.4 | 14.4 | — | — | 5.5 | 10.7 | 4.8 | 19.3 | |
| 2 | 70.3 | 60.2 | 63.2 | 61.4 | 63.0 | 83.7 | 70.5 | 84.2 | 83.9 | 75.2 | 69.3 | 62.3 | 60.2 | 81.1 | 72.3 | 81.1 | 72.3 | 83.0 | 2.3 | |
| 3 | 1.7 | 2.0 | 1.6 | — | — | 1.4 | 2.5 | 0.9 | — | 4.1 | 2.8 | 0.7 | 1.0 | 3.0 | 2.2 | 3.0 | 2.2 | 1.7 | 0.8 | |
| Total | 83.9 | 77.4 | 79.5 | 79.2 | 81.9 | 87.8 | 82.2 | 90.5 | 86.5 | 92.1 | 80.5 | 78.4 | 81.6 | 80.6 | 85.2 | 80.6 | 85.2 | 92.3 | 72.1 | |
| Mean age in years* | 2.03 | 1.92 | 1.96 | 1.92 | 1.93 | 2.10 | 2.13 | 2.09 | 2.09 | 2.05 | 2.01 | 2.00 | 1.00 | 2.13 | 2.04 | 2.12 | 2.04 | 2.12 | 1.91 | |

* On assumption that the type B growth = $\frac{1}{3}$ year + = trace (less than 0.05%)

Table 5. Comparison of present results with those of previous investigations as regards the distribution of the various smolt types in the various smolt classes. (As percentages).

| Rivers | Year or Years investigated | Reference | Smolt types | | | | | | |
|------------------|----------------------------|----------------------|---------------------|-----|-----|---------------------|------|-----|-----|
| | | | A | | | B | | | |
| | | | Smolt class (years) | | | Smolt class (years) | | | |
| | | | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Liffey ... | 1933-35 | Frost and Went, 1940 | 24.4 | 2.4 | — | 12.2 | 61.0 | — | — |
| | 1940-41 | Went, 1946 | 15.3 | 1.3 | — | 16.6 | 65.2 | 1.6 | — |
| | 1945 | Present results | 20.3 | 2.3 | — | 15.2 | 60.2 | 2.0 | — |
| Kerry Blackwater | 1943 | Unpublished data | 13.2 | 1.3 | — | 1.3 | 84.2 | — | — |
| | 1945 | Present results | 11.7 | 0.5 | — | 2.7 | 83.7 | 1.4 | — |
| Waterville | 1941 | Went and Barker 1943 | 13.4 | 5.0 | — | 5.1 | 72.6 | 3.9 | — |
| | 1945 | Present results | 13.8 | 4.0 | — | 3.2 | 76.5 | 2.5 | — |
| Shannon* ... | 1941 | Went, 1943 | 14.7 | 3.5 | 0.2 | 11.5 | 68.6 | 1.5 | — |
| | 1944 | Unpublished data | 10.2 | 9.6 | — | 8.2 | 68.7 | 3.3 | — |
| | 1945 | Present results | 5.8 | 2.1 | — | 12.8 | 75.2 | 4.1 | — |
| Corrib ... | 1924-26 | Went, 1943 | 14.6 | 8.6 | 0.4 | 11.8 | 60.6 | 3.9 | 0.1 |
| | 1945 | Present results | 6.8 | 3.5 | 0.2 | 17.4 | 69.3 | 2.8 | — |
| Ballisodare | 1938 | Went, 1941 | 32.7 | 0.8 | — | 22.9 | 42.5 | 1.1 | — |
| | 1939 | „ „ | 41.6 | 0.6 | — | 18.8 | 38.7 | 0.3 | — |
| | 1945 | Present results | 16.2 | 2.2 | — | 14.4 | 66.2 | 1.0 | — |
| Erne ... | 1938 | Went, 1942 | 15.1 | 2.9 | — | 4.8 | 76.9 | 0.3 | — |
| | 1945 | Present results | 5.6 | 2.1 | — | 4.8 | 83.0 | 4.5 | — |

* Results given only relate to the river since the advent of the hydro-electric scheme.

Table 6. Distribution of the different age groups (as percentages of the total catch in the various rivers.

| River | Age group (in winters) | | | | | | With SMS |
|-----------------------|------------------------|------|------|------|-----|-----|-------------|
| | 1+ | 2 | 2+ | 3 | 3+ | 4 | |
| Lifey . .. | 12.2 | 47.0 | 16.0 | 22.1 | — | 0.4 | 2.3 |
| Cork Blackwater . | 6.4 | 64.2 | 19.8 | 4.8 | — | 1.6 | 3.2 |
| Lee . | 3.8 | 90.0 | 3.4 | 1.8 | — | — | 1.0 |
| Bandon ... | 34.8 | 36.5 | 18.6 | 3.4 | 0.8 | — | 5.9 |
| Kerry Blackwater | 90.3 | 1.7 | 4.0 | — | — | — | 4.0 |
| Waterville ... | 6.0 | 81.7 | 3.3 | 1.7 | — | — | 7.3 |
| Laune | 37.4 | 48.1 | 8.1 | 1.0 | — | — | 5.4 |
| Feale ... | 26.8 | 62.5 | 6.5 | 0.3 | — | — | 3.9 |
| Shannon .. | 65.8 | 19.4 | 7.0 | 2.1 | — | — | 5.7 |
| Corrib | 41.5 | 31.1 | 15.8 | 0.4 | — | — | 11.2 |
| Moy | 36.0 | 35.0 | 20.5 | 0.3 | — | — | 8.2 |
| Ballisodare .. | 72.9 | 15.9 | 8.2 | 0.1 | — | — | 2.9 |
| Sligo | 1.1 | 92.1 | 1.3 | 0.7 | — | — | 4.8 |
| Bundrowes .. | 37.1 | 45.4 | 5.0 | 0.8 | 0.3 | — | 11.4 |
| Erne . | 50.2 | 7.1 | 25.2 | 1.7 | 0.2 | 0.2 | 15.4 |
| Boyne ... | 1.7 | 45.2 | 37.7 | 14.1 | 0.4 | 0.3 | 0.6 |
| All rivers combined . | 33.9 | 42.6 | 14.3 | 3.7 | 0.1 | 0.1 | 5.4 |

Table 7. Comparison of present results with those of previous investigations as regards the distribution of the various age groups. (As percentages).

| River | Year or years investigated | Reference | Age group (in winters) | | | | | | |
|------------------|----------------------------|-----------------------|------------------------|------|------|------|-----|-----|-----------|
| | | | 1+ | 2 | 2+ | 3 | 3+ | 4 | With SMS. |
| Liffey .. | 1940-41 | Went, 1946 | 14.7 | 49.0 | 13.1 | 20.8 | 0.1 | — | 2.3 |
| | 1945 | Present results | 12.2 | 47.0 | 16.0 | 22.1 | — | 0.4 | 2.3 |
| Kerry Blackwater | 1943 | Unpublished data | 86.7 | 4.2 | 6.7 | — | — | — | 2.4 |
| | 1945 | Present results | 90.3 | 1.7 | 4.0 | — | — | — | 4.0 |
| Waterville | 1941 | Went and Barker, 1943 | 32.9 | 55.4 | 2.5 | 2.0 | — | — | 7.2 |
| | 1945 | Present results | 6.0 | 81.7 | 3.3 | 1.7 | — | — | 7.3 |
| Shannon* .. | 1941 | Went, 1943 | 75.0 | 9.0 | 9.6 | 0.7 | — | 0.1 | 5.6 |
| | 1944 | Unpublished data | 74.2 | 15.6 | 4.6 | 2.1 | 0.1 | — | 3.4 |
| | 1945 | Present results | 65.8 | 19.4 | 7.0 | 2.1 | — | — | 5.7 |
| Corrib ... | 1924-26 | Went, 1943 | 61.4 | 16.6 | 14.7 | 0.9 | 0.4 | — | 6.0 |
| | 1945 | Present results | 41.5 | 31.1 | 15.8 | 0.4 | — | — | 11.2 |
| Ballisodare | 1938 | Went, 1941 | 81.3 | 6.3 | 6.0 | — | — | — | 6.4 |
| | 1939 | " " | 82.6 | 6.3 | 5.4 | 0.2 | — | — | 5.5 |
| | 1945 | Present results | 72.9 | 15.9 | 8.2 | 0.1 | — | — | 2.9 |
| Erne ... | 1928 | Went, 1942 | 58.7 | 7.5 | 25.2 | 1.7 | 0.5 | — | 6.4 |
| | 1945 | Present results | 50.2 | 7.1 | 25.2 | 1.7 | 0.2 | 0.2 | 15.4 |

* Results given only relate to the river since the advent of the hydro-electric scheme

Table 8. Years in which fish were hatched (as percentages of the total for the year).

| River | Years in which fish were hatched | | | | | | | |
|---------------------------|----------------------------------|------|------|------|------|------|------|------|
| | 1936 | 1937 | 1938 | 1939 | 1940 | 1941 | 1942 | 1943 |
| Liffey | — | — | — | 3.4 | 21.0 | 52.7 | 22.1 | 0.8 |
| Lee | — | — | — | 1.0 | 1.0 | 77.0 | 20.5 | 0.5 |
| Bandon | — | — | — | 2.5 | 5.9 | 42.4 | 45.8 | 3.4 |
| Kerry Blackwater .. | — | — | — | — | 0.1 | 9.9 | 88.3 | 1.5 |
| Waterville . | — | 0.4 | — | 3.3 | 8.0 | 80.0 | 8.3 | — |
| Laune . | — | — | 0.2 | 2.5 | 3.7 | 52.8 | 38.9 | 1.9 |
| Feale ... | — | — | — | 2.3 | 1.3 | 67.7 | 28.1 | 0.6 |
| Shannon | — | — | — | 1.6 | 2.5 | 28.4 | 60.9 | 6.6 |
| Coirib | — | — | — | 2.1 | 4.2 | 43.8 | 45.8 | 3.9 |
| Moy | — | — | 0.2 | 5.6 | 4.3 | 45.5 | 43.8 | 2.6 |
| Ballisodare . | — | — | — | 0.6 | 2.4 | 20.9 | 66.8 | 9.3 |
| Shigo .. | — | — | — | 0.2 | 12.1 | 81.3 | 5.3 | 1.1 |
| Bundrowes . | — | — | — | 7.5 | 5.0 | 46.0 | 38.2 | 3.3 |
| Erne . | 0.4 | — | — | 4.8 | 5.2 | 41.7 | 46.5 | 1.4 |
| Boyne ... | — | — | — | 0.7 | 12.9 | 71.3 | 15.1 | — |
| All rivers combined .. | + | + | + | 2.1 | 5.7 | 49.8 | 39.8 | 2.6 |

+ = trace (less than 0.05%).

Table 9. Comparison of present results with those of previous investigations as regards the distribution of the various "year groups" of salmon (As percentages of the total catch)

| River | Year or years investigated | Reference | Year group* | | | | | | | |
|------------------|----------------------------|-----------------------|-------------|---|-----|-----|------|------|------|------|
| | | | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 |
| Liffey | 1940-41 | Went, 1946 | — | — | 0.2 | 2.6 | 17.4 | 55.5 | 22.3 | 2.0 |
| | 1945 | Present results | — | — | — | 3.4 | 21.0 | 52.7 | 22.1 | 0.8 |
| Kerry Blackwater | 1943 | Unpublished data | — | — | — | 0.3 | 0.6 | 14.0 | 85.1 | — |
| | 1945 | Present results | — | — | — | — | 0.1 | 9.9 | 88.5 | 1.5 |
| Waterville .. | 1941 | Went and Barker, 1943 | — | — | 0.3 | 4.3 | 7.7 | 54.4 | 32.2 | 1.1 |
| | 1945 | Present results | — | — | — | 3.3 | 8.0 | 80.0 | 8.3 | — |
| Shannon† | 1941 | Went, 1943 | — | — | 0.4 | 1.3 | 3.6 | 19.3 | 67.8 | 7.6 |
| | 1944 | Unpublished data | — | — | 0.1 | 0.6 | 2.8 | 31.3 | 60.6 | 4.6 |
| | 1945 | Present results | — | — | — | 1.6 | 2.5 | 28.4 | 60.9 | 6.6 |
| Corrib .. | 1924-26 | Went, 1943 | — | — | 0.2 | 1.4 | 7.1 | 30.6 | 56.4 | 4.3 |
| | 1945 | Present results | — | — | — | 2.1 | 4.2 | 43.8 | 45.8 | 8.9 |
| Ballisodare .. | 1938 | Went, 1941 | — | — | — | 2.1 | 0.8 | 9.5 | 72.4 | 15.2 |
| | 1939 | | — | — | — | 0.2 | 1.3 | 9.6 | 76.4 | 12.7 |
| | 1945 | Present results | — | — | — | 0.6 | 2.4 | 20.9 | 66.8 | 9.3 |
| Erne ... | 1928 | Went, 1942 | — | — | 0.1 | 4.2 | 4.3 | 32.6 | 57.6 | 1.2 |
| | 1945 | Present results | 0.4 | — | — | 4.8 | 5.2 | 41.7 | 46.5 | 1.4 |

* Length of river and sea life combined

† Only observations since inception of hydro-electric scheme included.

Table 10. Absence habit and age at first spawning in the previous spawners.

| Absence habit | Age at first spawning (in winters) | | | | Total† |
|---------------------------------------|------------------------------------|--------------|-----------|----------|--------------|
| | 1+ | 2 | 2+ | 3 | |
| Short (less than a full year) | 190 | — | 8 | — | 211 (49.7%)† |
| Long (a full year) ... | — | 111 | — | 3 | 197 (46.3%)† |
| Very long (more than a full year) ... | 8 | — | 3 | — | 16 (3.8%)† |
| Total | 198 (61.1%) | 112* (34.6%) | 11 (3.4%) | 3 (0.9%) | 425†* |

† Including fish of which the age at first spawning could not be determined.

* Includes one S M 2 fish mentioned in text

No. 20.

SALMON OF THE KERRY BLACKWATER.

By

ARTHUR E. J. WENT

(Read NOVEMBER 26, 1946. Published separately May 21, 1947).

FRIAR O'SULLIVAN of Muckross Abbey in his *Ancient History of the Kingdom of Kerry* describes this river in the following terms. "The river Blackwater, the bounds 'twixt the parish of Templeoe and Ballybogg, noted for pearls and the salmon fishing in the Summer season, but deserves further notice that ye salmon goes on it two or three miles and within musket shot to the source thereof called Loch Brin, having in their way of said miles many cataracts and falls till coming to a smooth ford that has neither, which they never pass, nobody able to discover the secret. . ." (*Cork Hist. Soc. Jn.* 2nd Ser. 6, 149).

Cosmopolite in his book *The Sportsman in Ireland* (1840, 1, 130) refers to this river and states "The strictness with which this river has been preserved has rendered

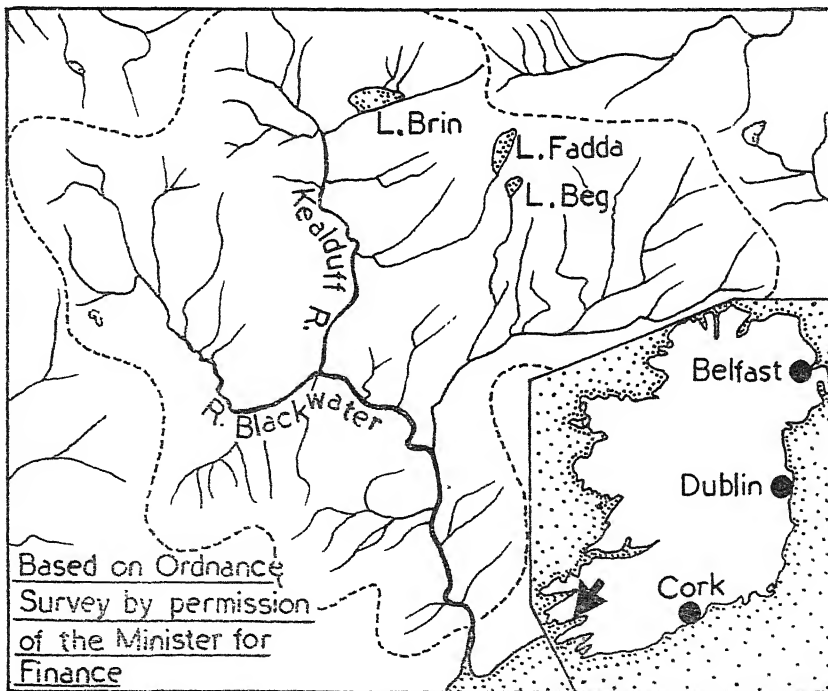


Fig 1. Sketch map of the catchment area of the Kerry Blackwater
(Arrow in inset map indicates location of the Kerry Blackwater)

nearly all the scattered inhabitants adepts in the art of fly-fishing." *The Parliamentary Gazetteer of Ireland* (1846, 1, 260) also describes the river picturesquely "It trots, tumbles and leaps noisily along a rugged path, at the bottom of a deep, copse-clad steep-sided ravine, and a little above its embouchure, rushes headlong beneath an aerially-poised bridge of two arches, spanning its yawning chasm. The stream has nearly as high attractions for the angler as for the poetic lover of landscape."

The Kerry Blackwater is a very small river with a catchment area of only 34 square miles and a length of approximately 10 miles. It drains some relatively high ground, some over 2,000 feet above sea level, and in consequence is a spate river. It has a number of small lakes on its watercourse and that of its tributaries, the most important of which is Lough Brin (see Fig. 1). The rocks of the catchment area belong mainly to the Old Red Sandstone, and therefore the waters thereof tend to be acid in character. There are adequate spawning grounds available, and it is noteworthy that the annual yield to the nets alone per square mile of the catchment area over the period 1875-1945, both years inclusive, has amounted to 43 salmon and peal, or about 300 lb. weight. Yields up to 105 salmon and peal per annum to the nets alone per square mile of catchment area, or about 740 lb. have been recorded. The Kerry Blackwater is a prolific river which affords first class angling when water conditions are satisfactory from the month of June onwards.

MATERIAL.

In 1943 a small collection of sets of scales and measurements (83 in number) of salmon and grilse (peal) taken in the nets in the estuary was made. In 1944 material was collected for the determination of the proportion of previously spawned fish in that river (see Went, 1945) and in 1945 further material, consisting solely of 595 sets of scales (without measurements), was collected from the fish taken by nets in the estuary. The possibility of obtaining more suitable material was remote, and therefore it was thought well to place the results of this limited examination on record.

SMOLT AGES.

Three smolt classes, one, two, and three years, were observed in the maiden fish. The bulk of the fish belonged to the two-year smolt class (see Table 1)

SMOLT TYPES

In previous publications on Irish salmon (Went, 1938-1946) it was mentioned that there were some fish which showed two or more annuli, corresponding to growth in the spring prior to the smolt migration. These were termed type B smolts. On the other hand there were fish which showed no such growth on their scales. For convenience these fish were termed type A smolts. In the present material the division has been carried out, and the results are given in Table 2.

AGE GROUPS.

In both 1943 and 1945 the fish were divided into three groups of maiden or unspawned fish and one heterogeneous group of previously spawned fish, having only one common character, namely the presence of spawning marks on their scales.

There were some fluctuations in the proportion of fish sampled throughout the season in 1943, so that the results were weighted against the catch figures. In 1945, however, sampling appeared to be adequate and representative, since the proportion of grilse calculated from the examination of scales was 90.3%, and that estimated from the visual examination of the fish was 89.3%. The weighted figures for 1945 have, however, been given in the various tables.

The grilse formed about nine-tenths of the total catch in both 1943 and 1945. None of the remaining age groups exceeded 7.0% of the total catch by numbers (see Table 3). In April a few summer fish ran into the river, and thereafter they predominated (see Table 4). In 1945 the bulk of the small spring fish ran before the end of May, as did almost half of the small summer fish. The greatest runs of grilse and previously spawned fish occurred in June (see Table 5). No information under this heading could be obtained directly for the year 1943 but all evidence available suggests that a similar condition prevailed during that year. Previously spawned fish comprised 2.4% of the total catch in 1943, 5.1% in 1944 (see Went, 1945) and 4.0% in 1945, respectively.

In Table 6 the years in which the various fish were hatched have been indicated. As should be obvious from Tables 1 and 3 one year class (i.e. the brood of one year) forms almost nine-tenths of the total catch. This fishery depends, therefore, on the spawning season $3\frac{1}{2}$ years earlier, i.e. in 1945 on the spawning season of 1941-42. If this was the only factor operating in determining the abundance of salmon in this fishery one would expect to find a good season followed by other good seasons at regular intervals. Similarly poor seasons should follow one another at the same interval, but such is not the case as will be seen later.

SIZE DISTRIBUTION.

The size distribution, as based on the limited material from 1943, has been given in Table 7. It will be seen that just over 70% of the total catch had lengths between 23.95 and 27.95 inches.

AVERAGE SIZES.

The average sizes in 1943 in the different age groups have been given in Table 8. It must be acknowledged that the material is very limited, but the results so indicated do not call for any comment. It is obvious that the Kerry Blackwater is a small fish river. Col. E. Hood informs me that a fish of 33 lb. weight was taken about 15 years ago on the rod, and about 6 years ago one of 29 lb. was taken in the nets, but these were exceptional fish. Most of the fish, in fact, are under 10 lb. in weight.

CONDITION CO-EFFICIENT.

The method of determining the condition co-efficient (or condition factor) has been given in an earlier paper (Went, 1941), and the mean values in the various age groups in 1943 are given in Table 9. With an average value of 1.14 on Menzies Scale the condition of these fish is good, being similar to that of salmon of other Irish rivers (see Went, 1938-1946).

Table 1. Distribution of the various smolt ages.

| Smolt Age | 1943 | | 1945 | | Combined Results | |
|-----------|------|-------|------|-------|------------------|-------|
| | No. | % | No. | % | No. | % |
| 1 | 1 | 1.3 | 16 | 2.7 | 17 | 2.6 |
| 2 | 74 | 97.4 | 544 | 95.4 | 618 | 95.5 |
| 3 | 1 | 1.3 | 11 | 1.9 | 12 | 1.9 |
| Total | 76 | 100.0 | 571 | 100.0 | 647 | 100.0 |

Table 2. Distribution of the various smolt types.

| Smolt Age | Smolt type | | | | Both types | |
|--------------|------------|------|-----|------|------------|-------|
| | A | | B | | | |
| | No. | % | No. | % | No. | % |
| 1943 | | | | | | |
| 1 | - | | 1 | 1.3 | 1 | 1.3 |
| 2 | 10 | 13.2 | 64 | 84.2 | 74 | 97.4 |
| 3 | 1 | 1.3 | - | - | 1 | 1.3 |
| Total | 11 | 14.5 | 65 | 85.5 | 76 | 100.0 |
| 1945 | | | | | | |
| 1 | - | - | 16 | 2.7 | 16 | 2.7 |
| 2 | 66 | 11.7 | 478 | 83.7 | 544 | 95.4 |
| 3 | 3 | 0.5 | 8 | 1.4 | 11 | 1.9 |
| Total | 69 | 12.2 | 502 | 87.2 | 571 | 100.0 |

Table 3. Distribution of the various age groups (as percentage of the total catch).

| Age Group (in winters) | 1943 | 1945 |
|------------------------|-------|-------|
| 1+ | 86.7% | 89.2% |
| 2 | 4.2% | 2.1% |
| 2+ | 6.7% | 4.7% |
| With S.Ms. | 2.4% | 4.0% |

Table 4. Proportion of spring and summer fish in the 1943 and 1945 seasons.

| Month | 1943 | | 1945 | |
|--------------|---------------|-------------|---------------|-------------|
| | Proportion of | | Proportion of | |
| | Spring Fish | Summer Fish | Spring Fish | Summer Fish |
| April | 75.0% | 25.0% | - | - |
| May | 42.8% | 57.2% | 33.3% | 66.7% |
| June | 6.3% | 93.7% | 0.9% | 99.1% |
| July | Nil | 100.0% | Nil | 100.0% |
| August | Nil | 100.0% | - | - |
| Whole Season | 5.1% | 94.9% | 1.7% | 98.3% |

Table 5. The proportion of each age group in each month (as percentage of total catch) in 1945.

| Month | Age group (in winters) | | | | Total |
|-----------|------------------------|-------|-------|------------|-------|
| | 1+ | 2 | 2+ | With S.Ms. | |
| April/May | 0.8 | 76.2 | 49.0 | 5.0 | 4.8 |
| June | 59.5 | 23.8 | 44.6 | 52.5 | 57.8 |
| July | 39.7 | - | 6.4 | 42.5 | 37.4 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

Table 6. Divided migration and return (as percentage total catch). Note:- These figures have not been weighted against catch figures.

| Returned in 1945 as | Hatched in | | | | Total |
|---------------------|------------|------|------|------|-------|
| | 1940 | 1941 | 1942 | 1943 | |
| 1 winters | - | 1.7 | 87.1 | 1.5 | 90.3 |
| 2 winters | - | 1.2 | 0.5 | - | 1.7 |
| 2 winters | 0.1 | 3.2 | 0.7 | - | 4.0 |
| With S.Ms. | - | 3.8 | 0.2 | - | 4.0 |
| Total | 0.1 | 9.9 | 88.5 | 1.5 | 100.0 |

Table 7. Size distribution as percentage of total catch.

| Class Interval* | Age Group (in winters) | | | | Total |
|-----------------|------------------------|-----|-----|------------|-------|
| | 1+ | 2 | 2+ | With S.Ms. | |
| 18 | 2.0 | - | - | - | 2.0 |
| 20 | 4.0 | - | - | - | 4.0 |
| 22 | 7.9 | - | - | - | 7.9 |
| 24 | 49.6 | 0.1 | - | - | 49.7 |
| 26 | 21.7 | 0.2 | - | - | 21.9 |
| 28 | 4.0 | 0.9 | 0.7 | 0.7 | 6.3 |
| 30 | - | 0.5 | 2.7 | - | 3.2 |
| 32 | - | 0.2 | 1.0 | 2.0 | 3.2 |
| 34 | - | 0.2 | 0.3 | 1.3 | 1.8 |
| Total | 89.2 | 2.1 | 4.7 | 4.0 | 100.0 |

* Class interval 18 etc. includes all fish having lengths between 17.95 and 19.95 inches etc.

Table 8. Mean, minimum and maximum weights and lengths in the various age groups.

| Age Group (in winters) | Length in inches | | | Weight in lb. | | |
|------------------------|------------------|------|------|---------------|------|------|
| | Mean | Min. | Max. | Mean | Min. | Max |
| 1+ | 25.2 | 19.5 | 28.1 | 6.6 | 2.0 | 10.0 |
| 2 | 29.7 | 25.5 | 32.3 | 10.9 | 6.5 | 15.0 |
| 2+ | 31.3 | 28.0 | 34.5 | 12.6 | 9.0 | 16.5 |
| With S.Ms. | 32.7 | 28.5 | 35.0 | 15.0 | 12.0 | 18.0 |
| Total | - | 19.5 | 35.0 | - | 2.0 | 18.0 |

Table 9. The mean condition coefficients and condition factors in the various age groups in 1943.

| Age Group (in winters) | Mean condition coefficient (K) | Mean condition factor (C.F.) |
|------------------------|------------------------------------|------------------------------|
| | $K = \frac{W}{L^3} \times 0.00036$ | $C.F. = \frac{W}{L^3}$ |
| 1+ | 1.14 | 41.2 |
| 2 | 1.15 | 41.5 |
| 2+ | 1.14 | 41.2 |
| With S.Ms. | 1.19 | 43.1 |
| Total | 1.14 | 41.2 |

W = Weight L = Length

Table 10. Fluctuations in the catches in the Kerry Blackwater and Ballisodare Rivers and the export figures (as percentages of the mean values for the period 1924 to 1945, both years inclusive)

| Year | Percentages of mean for the period 1924 to 1945 | | |
|------|---|-------------------|--|
| | Kerry Blackwater | Ballisodare River | Exports in cwt. June/October from Bire |
| 1924 | 25 | 90 | 124 |
| 1925 | 119 | 99 | 122 |
| 1926 | 86 | 93 | 143 |
| 1927 | 75 | 63 | 117 |
| 1928 | 55 | 46 | 79 |
| 1929 | 9 | 41 | 54 |
| 1930 | 89 | 124 | 109 |
| 1931 | 180 | 138 | 128 |
| 1932 | 107 | 105 | 120 |
| 1933 | 112 | 105 | 93 |
| 1934 | 134 | 123 | 156 |
| 1935 | 83 | 122 | 114 |
| 1936 | 116 | 97 | 92 |
| 1937 | 50 | 80 | 42 |
| 1938 | 39 | 57 | 44 |
| 1939 | 120 | 110 | 78 |
| 1940 | 247 | 115 | 86 |
| 1941 | 221 | 172 | 166 |
| 1942 | 158 | 160 | 133 |
| 1943 | 147 | 109 | 106 |
| 1944 | 160 | 95 | 63 |
| 1945 | 91 | 66 | 32 |

Table 11. Growth in freshwater.

| Smolt Age | Number Examined | Length in inches at end of | | | Smolt Length in ins. |
|-----------|-----------------|----------------------------|----------|----------|----------------------|
| | | 1st Year | 2nd Year | 3rd Year | |
| 1 | 1 | 3.6 | — | — | 6.4 |
| 2 | 74 | 2.0 | 4.3 | — | 4.9 |
| 3 | 1 | 1.0 | 3.4 | 4.7 | 4.7 |

Table 12. Mean lengths at the end of the different sea winters.

| Age Group (in winters) | Number Examined | Length in inches at end of | |
|------------------------|-----------------|----------------------------|----------------|
| | | 1st Sea Winter | 2nd Sea Winter |
| 1+ | 45 | 20.0 | — |
| 2 | 12 | 18.4 | 29.7 |
| 2+ | 19 | 18.6 | 29.2 |

Table 13. The annual catches of salmon (and grilse) in the Kerry Blackwater from 1875 to 1945, both years inclusive. (As percentages of the mean annual yield from 1875 to 1945).

| | | | | | | | | | | | |
|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------------|
| Year Catch (%) | 1875 128 | 1876 180 | 1877 182 | 1878 88 | 1879 68 | 1880 188 | 1881 147 | 1882 21 | 1883 118 | 1884 95 | 1875/84 122 |
| Year Catch (%) | 1885 135 | 1886 170 | 1887 149 | 1888 202 | 1889 177 | 1890 148 | 1891 242 | 1892 134 | 1893 147 | 1894 267 | 1885/94 157 |
| Year Catch (%) | 1895 176 | 1896 141 | 1897 119 | 1898 114 | 1899 216 | 1900 98 | 1901 90 | 1902 194 | 1903 91 | 1904 25 | 1895/1904 126 |
| Year Catch (%) | 1905 24 | 1906 38 | 1907 41 | 1908 95 | 1909 158 | 1910 99 | 1911 50 | 1912 37 | 1913 56 | 1914 60 | 1905/14 66 |
| Year Catch (%) | 1915 64 | 1916 35 | 1917 88 | 1918 61 | 1919 98 | 1920 30 | 1921 45 | 1922 34 | 1923 75 | 1924 21 | 1915/24 55 |
| Year Catch (%) | 1925 102 | 1926 74 | 1927 64 | 1928 47 | 1929 8 | 1930 76 | 1931 147 | 1932 92 | 1933 96 | 1934 115 | 1925/34 69 |
| Year Catch (%) | 1935 71 | 1936 100 | 1937 45 | 1938 33 | 1939 105 | 1940 212 | 1941 189 | 1942 135 | 1943 126 | 1944 137 | 1935/45 106 |

1945 - 78%

CALCULATED LENGTHS

The length of each unspawned or "maiden" fish at the end of every year of its life was calculated in the usual way from measurements of the scales, i.e., assuming that the growth of the fish is strictly proportional to the growth of the scale.

River Life. In this section the growth in fresh water will be discussed. Owing to the limited number of observations only the results for the two-year smolt class are significant (see Table 11). The growth rate in the two-year smolt class is similar to that of the same smolt class in the Ballisodare River (see Went, 1941).

Sea Life. The mean lengths at the end of the first and second sea winters have been indicated in Table 12. The grilse appear to be longer on an average than any other age group at the end of the first sea winter. There is slight differentiation in the lengths at the end of the first and second sea winters in the small spring and summer fish, the numbers involved are, however, very small.

FLUCTUATIONS IN THE YIELD OF THIS FISHERY.

The records of the catches by nets in the Kerry Blackwater are available from the year 1875, and these have been given in Table 13 (as percentages of the average annual catch over the period 1875-1945, both years inclusive). In the first 35 years a draft net and a sweeper net* were used in the fishery, but about 1910 the sweeper net was discontinued owing to the small yields then prevailing. It will be noticed from Fig. 2 that there were considerable fluctuations in the yield. No doubt some of these can be partly attributed to different climatic conditions in the different years. To eliminate the effects of the climatic conditions the 10 years averages since 1875 were calculated, and a smoothed curve showing the results has been added to Fig. 2.

* A form of seine net used with two boats (see "The Irish Pilchard Fishery," Went, 1946.)

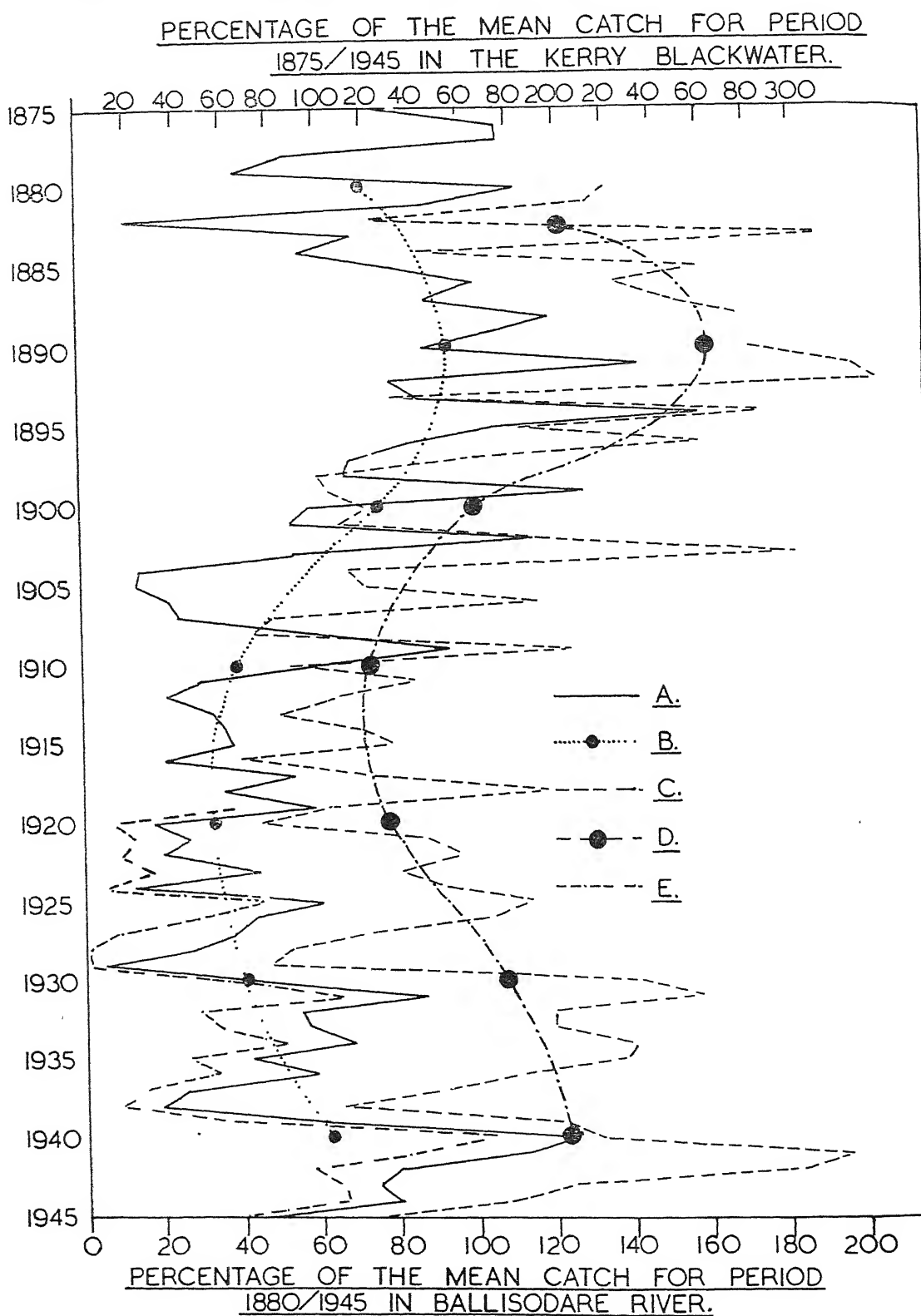


Fig. 2. Fluctuations in the catches in the Kerry Blackwater and Ballisodare River. A=Catches in Kerry Blackwater; B=Smooth curve for Kerry Blackwater based on ten-year averages; C=Catches in Ballisodare River, D=Smooth curve for Ballisodare River based on ten-year averages, E=Catches of grilse in Kerry Blackwater.

This shows that the annual yield increased slightly from 1875/84 to 1885/94 and then decreased until 1915/24, thereafter increasing to date. The latest period, however, has an average yield somewhat less than the 1895/1904, but of course it must be remembered that in the former period a sweeper net had been used. To obtain completely comparable figures an estimate of the yield of the sweeper net would have to be added, in which case the yields in the period 1935/45 would probably exceed those of 1895/1904. There is no reason to believe that there is a permanent decline in the fishery when compared with the early part of the period under review.

From 1920 the actual catches of grilse were recorded and these have been indicated in Fig. 2. It will be seen that the bulk of the fish in the catches were, in fact, grilse.

Records of catch figures in fisheries, such as these, are of great value. It is interesting to compare the present catch figures with those of another river (the Ballisodare River) of the same character as the Kerry Blackwater. In Table 10 the annual figures for both rivers have been indicated as percentages of the mean annual catch from 1924 to 1945. For the sake of comparison some other returns have been added. These are the exports in cwt. from the month of June onwards from Eire since 1924. As mentioned elsewhere (Went, 1938) the exports from June onwards may be regarded as summer fish, and the export returns probably give a reasonably accurate idea of the fluctuations of the quantities of summer fish captured. These export figures are included in *Trade Statistics*, published monthly by the Department of Industry and Commerce, Dublin. Unfortunately we only have the weights (and not numbers), but the export figures for the period may be considered as reasonably comparable. These returns have been plotted in Fig. 3. Generally there is close agreement in the three curves, the most obvious differences being in the export figures for 1926 and the Kerry Blackwater for 1940 and 1944. Whereas the exports and the catches in the Ballisodare River reached a maximum in 1941, this occurred a year earlier in the Kerry Blackwater. It is also noteworthy that in 1927 the catches in the Kerry Blackwater and the Ballisodare Rivers were somewhat below average, whereas in "spring fish" rivers throughout Europe 1927 was a peak year. Grilse (peal) were not particularly plentiful in Ireland in 1927.

Referring again to Fig. 3 it will be seen that the catches in the Kerry Blackwater showed much greater extremes in fluctuations (from 9% in 1929 to 247% in 1940) than were noticed in either the Ballisodare River (41% in 1929 to 172% in 1941) or in the exports (32% in 1945* to 166% in 1941). Why might we ask are there bigger fluctuations in the Kerry Blackwater than in the other two cases? A complete answer to this question cannot be given but as mentioned earlier about 90% of the fish of any particular year in the Kerry Blackwater have been derived from the brood resulting for a single spawning season. If for any reason the spawning season was bad, a poor yield would probably result after the appropriate interval,

* The proportion of salmon consumed at home was certainly greater in 1945 than in former years so that there is a tendency for the shortage in that year to be over-emphasised

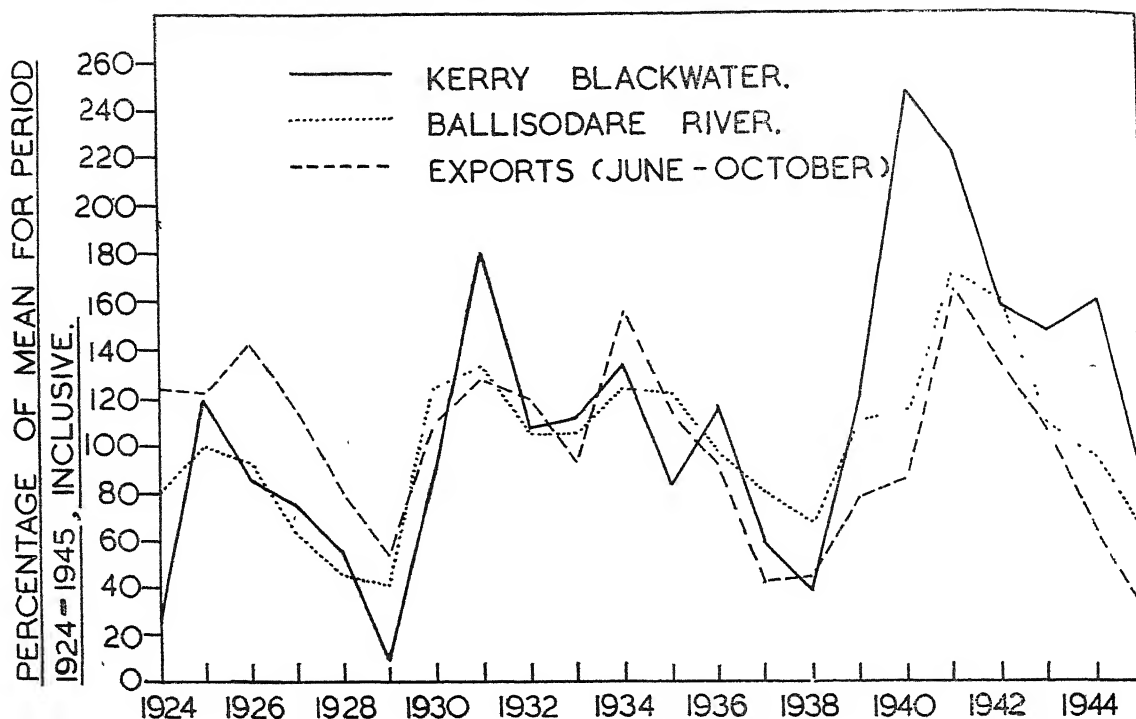


Fig 3 Fluctuations in the catches in the Kerry Blackwater and Ballisodare River and in the exports of salmon from Eire from June onwards

and similarly the results of an excellent spawning season would be reflected in the catch of a single year. In the Ballisodare River and in many other fisheries in Ireland there is a tendency for the catch to be spread over two or more brood years (see Went, 1938 to 1946) In other words the "safeguards" of "divided migration and return" are more or less eliminated in a river of the class of the Kerry Blackwater, the "eggs all being in one basket" as it were

Let us now compare the catches in the Kerry Blackwater with those of the Ballisodare River over a longer period. The appropriate curve for the Ballisodare River has been added to Fig. 2. It will be seen that there is no complete agreement between the curves representing the catches in the Kerry Blackwater and the Ballisodare River over the whole period. If, however, one draws a smooth curve representing the ten-year averages (see Fig. 2) there is a marked similarity between the two sets of observations. In each case a maximum was reached in the period 1885-1894 and a minimum in the period 1905-1925, that for the Ballisodare being somewhat earlier than that for the Kerry Blackwater. From about 1920 onwards there has been a marked increase. It is, therefore, difficult not to accept the conclusion that the catches (and presumably the runs) tend to show some periodicity, and that these fluctuations are independent of artificial alterations produced by man's activities.

The writer wishes to take this opportunity of expressing his thanks to Colonel E. Hood, Dromore Castle, Kenmare, County Kerry, for his kindness in providing the material and data on which this investigation was based.

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No. 21.

EFFECT OF MILD STRAINS OF VIRUS X ON THE YIELD OF
UP-TO-DATE POTATO.

By

PHYLLIS E. M. CLINCH and ROBERT MCKAY.

(Department of Plant Pathology, University College, Dublin)

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INTRODUCTION.

ALTHOUGH virus X is the commonest virus attacking potatoes, there has been comparatively little investigation into the effects of this virus on the cropping capacity of the plants. This may be largely due to the fact that many of the most popular commercial varieties, especially in America, are not known in an X-free condition, and therefore the problem has not had a widespread practical significance.

A survey of potato crops in Australia by Bald and Pugsley (2) revealed that the principal commercial varieties grown there were permeated with various strains of virus X. In the course of his investigations, however, Bald (1) discovered a small number of X-free plants of the widely grown Up-to-Date or Factor variety. It then became of interest to determine whether the expected increase in yield of the healthy plants over those which were carriers of X would justify the expenditure necessary to propagate these X-free stocks. The results of a number of experiments extending from 1940 onwards led Bald (3) to the conclusion that the presence of even a masked strain of X causes a significant reduction in yield of tubers, and the policy of multiplying X-free stocks was consequently adopted.

In 1941, Scott (10) stated that the presence of mosaic in Scottish-grown potatoes caused a reduction in yield which varied from 13.3% to 67.3% depending upon the severity of the symptoms; but the diseases studied were not confined to those caused by virus X alone. In 1944, Schultz and Bonde (9) reported that latent mosaic (virus X) reduced the yield of certain American varieties of potato by 9—22%. In the same year, Smith and Markham (11) estimated the comparative yields of healthy and X-infected plants of 9 different varieties of potato at Cambridge, and found a reduction in yield due to the virus of 12%.

In this country, virus-free stocks of such popular varieties as Kerr's Pink, British Queen, Great Scot, Majestic, Arran Banner, Eclipse, etc., have been tested and maintained at the Albert College, Glasnevin, for many years. These nucleus stocks serve as the basis of the foundation seed potato stocks propagated by the Department of Agriculture in accordance with a scheme initiated in 1924. The important variety Up-to-Date was not, however, available in an X-free condition

until recently, when, following the announcement by Bald that X-free stocks existed in Australia, a further search was made in Ireland, and two X-free plants were found in 1943. As the bulk of the Up-to-Date grown in this country is a carrier of X, and is yet on the average one of our best yielding varieties, it was considered of interest to determine to what extent X-free Up-to-Date plants would outyield carrier plants under our conditions. The infection and propagation of the material required two years, so that figures for only one trial are available so far. As these show a somewhat unexpected trend, however, it was decided to furnish a report on the work already carried out.

EXPERIMENTAL PROCEDURE.

In order to obviate the effects of possible agronomic differences between clones, and also with a view to studying the effects of such differences should they exist, it was decided to propagate all the experimental material from single tubers of the two available clones of Up-to-Date. It was also decided, for various reasons, that the infected plants in the trial should be grown from infected tubers rather than inoculated during the trial season, one reason being the difficulty of achieving uniform systemic infection in field inoculated plants. Experience with a severe strain of X has shown that, even when plants are not more than 6 inches above ground at the time of inoculation, individual shoots frequently escape the initial infection, and several weeks may elapse before they contract the disease.

Accordingly, in the spring of 1944 two large X-free tubers were selected, each of which could be cut into 3 sets. The 6 sets were planted in 12 in. pots in an insect-proof glasshouse. One of the plants of each clone was kept as a healthy control, while the remaining two in each case were grafted with scions of Up-to-Date carrying mild strains of virus X. The scions grew vigorously, and the plants were allowed to mature fully before harvesting the tubers. In this way, systemic infection of the tubers was ensured.

Two strains of virus X were employed, both naturally occurring types in Up-to-Date. They will be referred to as strains (a) and (b), respectively, for the purpose of the present paper. Strain (a) may be regarded as a typical mild strain of X. It is carried symptomlessly by Up-to-Date and all other common British varieties, excepting such X-intolerant ones as Epicure and King Edward. In *Datura stramonium* and in tobacco var. White Burley it causes a barely discernible light and dark green mottling. The disease was identified as "mottle" in American terminology by Koch and Johnson (8) in 1935. Strain (b) is that commonly present in Up-to-Date, and was formerly known as "Up-to-Date Streak" and later as virus B (5). It is carried symptomlessly by Up-to-Date, but when artificially transmitted by grafting it induces top-necrosis in a number of varieties not so affected by typical strains of X. Apart from the latter reaction, however, it behaves as a mild strain of X (6). In those varieties which are tolerant to it it is carried symptomlessly as in Up-to-Date. In *Datura stramonium* and in White Burley tobacco the respective symptoms are a mild mosaic accompanied by slight necrosis and a nebulous ringspot. Koch and Johnson (8) identified the virus content of this Up-to-Date stock as "mottle and ringspot"; and it is also the opinion of Cockerham (7) that

the material contains a mixture of the top-necrosis strain with a typical mild X strain. For practical purposes, however, the two Up-to-Date sources are carriers of mild strains of X which are distinct from one another although similar in their reactions on Up-to-Date. Both stocks of Up-to-Date have been maintained under glass at Glasnevin for over 20 years as part of the stock laboratory cultures.

In 1945 the produce of the 6 units was grown in a small isolated plot situated amongst beans in a freshly ploughed pasture field. Six tubers of each experimental unit and six of each Up-to-Date source of X were planted in short drills, separated from one another and bounded by drills of X-immune seedling 41956. The drills were manured in the ordinary way with farmyard manure. The plants grew uniformly and were sprayed twice during the season with 2% Burgundy mixture. On July 20th, the plants were tested by inoculation to *Datura stramonium*, and it was found that virus X was present only in those plants which had been grafted with infected scions in the previous year. The tubers were harvested on October 5th, the dead tops having been cut away two weeks previously. Although this plot was intended for purposes of propagation, the yields of tubers obtained from the different units of the two clones are recorded in Table 1, and will be discussed later.

TABLE 1.—YIELDS OF HEALTHY AND X-INFECTED UP-TO-DATE PLANTS, 1945.

| | | | | Total yield of 6 plants. | Average yield per plant. | Yield as percentage of healthy. |
|-------------|---------------------|-----|-----|-----------------------------|-----------------------------|---------------------------------------|
| Clone I | Healthy | ... | ... | 24 lb. 6 ozs. | 4 lb. 1 oz. | 100 |
| | Infected Strain (a) | ... | ... | 24 „ 8 „ | 4 „ 1 „ | 100 |
| | Infected Strain (b) | ... | ... | 31 „ 11 „ | 5 „ 4 „ | 130 |
| Clone II | Healthy | ... | ... | 32 „ 0 „ | 5 „ 5 „ | 100 |
| | Infected Strain (a) | .. | .. | 32 „ 10 „ | 5 „ 7 „ | 101.9 |
| | Infected Strain (b) | ... | ... | 35 „ 8 „ | 5 „ 15 „ | 110.9 |

YIELD TRIAL 1946.

The majority of tubers obtained from the propagation plot were of ware size and included some very large tubers. The use of whole sets of equal size throughout the trial was impracticable; cut sets of equal weight were also excluded as it was considered undesirable that any tuber should be cut into more than two portions. The tubers of each healthy clone were therefore graded according to weight and selections made from them in such a way that an equal number of tubers of similar weight was available in the corresponding infected units of the clone. Hence, although the weights of the tuber sets varied in any one lot, the variation was uniform as between healthy and infected lots of the same clone. The weights of the different

grades of set were as follows Grade A=40—80 grms, Grade B=80—140 grms., Grade C=140—180 grms, and Grade D=180—470 grms. Tubers exceeding 180 grams in weight were cut in two longitudinally, and altogether there were 69 experimental sets of each lot. The numbers of tubers of each grade in the different clones are shown in Table 2

The experimental plot was situated in a level field which had carried a crop of beans in 1945 and had previously been in grass for 20 years. Each experimental lot was grown in a separate drill, the drills being 30 ins apart and the plants 18 ins. apart in the drill. The arrangement of the drills is shown below. There were eight experimental drills, including three drills (one healthy and two infected) of each clone, and a drill each of the Up-to-Date sources of the X strains (*a*) and (*b*). To obviate border effects three extra plants were grown at the ends of the drills, and these were neglected at digging time. In addition, a drill of healthy Majestic plants separated the healthy and the infected lots of each clone, while the plot was bordered on either side by two more drills of healthy plants, making in all 17 drills of 75 plants each. The remainder of the field was sown with a mixture of oats and legumes for ensilage.

ARRANGEMENT OF DRILLS IN FIELD PLOT, 1946.

1. Up-to-Date. Healthy.
2. Epicure Healthy.
3. Majestic. Healthy.
- 4.* Up-to-Date. Healthy. Clone I.
- 5.* Up-to-Date. Healthy. Clone II.
6. Majestic Healthy.
- 7.* Up-to-Date infected Strain (*a*). Clone I.
- 8.* Up-to-Date infected Strain (*a*). Clone II
9. Majestic. Healthy.
- 10.* Up-to-Date infected Strain (*b*). Clone I.
- 11.* Up-to-Date infected Strain (*b*). Clone II.
12. Majestic. Healthy.
- 13.* Up-to-Date. Source of Strain (*a*).
- 14.* Up-to-Date Source of Strain (*b*).
- 15 Majestic. Healthy.
16. Epicure. Healthy.
17. Up-to-Date. Healthy.

* Experimental drill.

The drills were manured with farmyard manure and the usual artificials and the sets planted on April 11th. On May 12th when most of the plants were above ground, a sharp frost occurred which caused scorching of the foliage in scattered plants throughout the plot. No plant was seriously injured, however, and following rain in the latter part of May the plants grew extremely vigorously. There had been no "misses," and there was a complete absence of root troubles such as

Blackleg (*Bacillus phytophthorus*). No difference whatever was apparent between the healthy and infected plants, and by the middle of June the uniformly healthy and vigorous appearance of all the experimental plants was remarkable. About the end of June, however, when the plants were in full flower, a conspicuous yellowish hue developed in the tops of 3 adjacent plants in Drill 11 (Clone II infected X strain (b)), and shortly afterwards the same chlorotic effect appeared in contiguous plants in the same drill. In these the yellowing appeared first in one or two shoots and then spread gradually through the plant, eight plants altogether showing definite chlorosis. Owing to the manner of its appearance and the fact that it was strictly confined to one spot, the effect was at first thought to be due to some unknown soil factor operating in this particular part of Drill 11. There was no trace of rolling in the lower leaves at first, but slight rolling did occur eventually, and in the meantime grafts of scions from the affected plants to healthy President potatoes demonstrated conclusively that the condition was primary leaf roll. A close watch was kept on the remaining drills, but no further leaf roll could be detected during the season, nor was there any difference in appearance between the healthy and X-infected plants generally.

On July 2nd, the plot was sprayed with 2% Burgundy mixture. At this period growth was luxuriant, but from July onwards the weather became abnormally wet with very little sunshine, and the plants were buffeted by wind and rain, especially by a severe rainstorm which occurred on August 12th. The unfavourable effect of such conditions on aphids probably explains why the leaf roll failed to spread from the initial point of infection. Blight (*Phytophthora infestans*) had not yet appeared at the time of the second spraying on July 29th, but began to develop towards the end of August, and became widespread during September. The application of a third spray in August was impossible owing to the tangled state and brittle nature of the stalks. Early in October the plants were completely dead, and harvesting took place on October 25th. The tubers were taken indoors and brushed by hand to remove loosely adhering soil before being weighed. The weights of ware, seed, and chits, in each grade were taken, as well as the total yield of tubers in each grade. Notwithstanding the severity of Blight attack on the foliage the number of tubers showing blight lesions was negligible. This was probably due to the fact that the onset of the disease was late and its progress rapid.

During the latter half of July the absence of virus X in plants of Drills 4 and 5 was confirmed by inoculation to *Datura stramonium*. The plants were tested in groups of five, a single leaf being taken from each plant. It had previously been ascertained that inoculum prepared by grinding up as many as nine healthy leaves with one infected one could be relied upon to produce symptoms of X in *Datura*. In addition, several scions were taken at random from the same drills, and these failed to produce symptoms when grafted on healthy Epicure potato in the glass-house.

TABLE 2
COMPARATIVE YIELDS OF HEALTHY AND X-INFECTED
UP-TO-DATE POTATO PLANTS 1946

| CLONE I | | | | | | | | | | |
|--|--------------|--------------|------|----|------|----|-------|----|-------------|--------------------------------|
| | Grade of set | No of plants | Ware | | Seed | | Chats | | Total yield | Yield as percentage of healthy |
| | | | lbs | oz | lbs | oz | lbs | oz | lbs oz | |
| Healthy | A | 12 | 29 | 6 | 9 | 3 | 1 | 3 | 39 12 | |
| | B | 17 | 45 | 8 | 13 | 2 | 2 | 9 | 61 3 | |
| | C | 14 | 39 | 12 | 9 | 0 | 2 | 11 | 51 7 | |
| | D | 26 | 55 | 14 | 21 | 4 | 4 | 0 | 81 2 | |
| Totals | | 69 | 170 | 8 | 52 | 9 | 10 | 7 | 233 8 | 100 |
| Infected Strain (a) | A | 12 | 30 | 15 | 5 | 9 | 0 | 4 | 36 12 | |
| | B | 17 | 35 | 8 | 14 | 2 | 4 | 0 | 53 10 | |
| | C | 14 | 35 | 8 | 12 | 6 | 2 | 15 | 50 13 | |
| | D | 26 | 59 | 0 | 21 | 12 | 5 | 8 | 86 4 | |
| Totals | | 69 | 160 | 15 | 53 | 13 | 12 | 11 | 227 7 | 97 |
| Infected Strain (b) | A | 12 | 35 | 5 | 4 | 4 | 0 | 7 | 40 0 | |
| | B | 17 | 39 | 8 | 16 | 4 | 9 | 2 | 64 14 | |
| | C | 14 | 32 | 9 | 16 | 12 | 7 | 2 | 56 7 | |
| | D | 26 | 80 | 10 | 18 | 0 | 4 | 10 | 103 4 | |
| Totals | | 69 | 188 | 0 | 55 | 4 | 21 | 5 | 264 9 | 113 |
| CLONE II. | | | | | | | | | | |
| Healthy | B | 11 | 32 | 0 | 7 | 7 | 2 | 1 | 41 8 | |
| | C | 4 | 10 | 4 | 4 | 10 | 1 | 8 | 16 6 | |
| | D | 54 | 153 | 10 | 27 | 10 | 12 | 7 | 193 11 | |
| Totals | | 69 | 195 | 14 | 39 | 11 | 16 | 0 | 251 9 | 100 |
| Infected Strain (a) | B | 11 | 22 | 10 | 5 | 8 | 1 | 6 | 29 8 | |
| | C | 4 | 11 | 0 | 3 | 14 | 1 | 0 | 15 14 | |
| | D | 54 | 176 | 15 | 32 | 0 | 8 | 3 | 217 2 | |
| Totals | | 69 | 210 | 9 | 41 | 6 | 10 | 9 | 262 8 | 104 |
| Infected Strain (b) | B | 11 | 45 | 12 | 2 | 12 | 1 | 9 | 50 1 | |
| | C | 4 | 9 | 0 | 5 | 2 | 1 | 7 | 15 9 | |
| | D | 54 | 100 | 10 | 33 | 2 | 16 | 0 | 149 12 | |
| Totals | | 69 | 155 | 6 | 41 | 0 | 19 | 0 | 215 6 | 86 |
| UP-TO-DATE SOURCES OF X STRAINS (a) AND (b). | | | | | | | | | | |
| Strain (a) | A | 12 | 26 | 14 | 5 | 10 | 0 | 15 | 33 7 | |
| | B | 17 | 34 | 9 | 18 | 8 | 5 | 13 | 58 14 | |
| | C | 14 | 29 | 5 | 16 | 2 | 4 | 14 | 50 5 | |
| | D | 26 | 68 | 15 | 21 | 3 | 8 | 14 | 99 0 | |
| Totals | | 69 | 159 | 11 | 61 | 7 | 20 | 8 | 241 10 | 103 |
| Strain (b) | A | 12 | 28 | 4 | 5 | 9 | 1 | 13 | 35 10 | |
| | B | 17 | 37 | 0 | 16 | 0 | 7 | 2 | 60 2 | |
| | C | 14 | 25 | 8 | 16 | 0 | 7 | 6 | 48 14 | |
| | D | 26 | 73 | 0 | 25 | 12 | 10 | 12 | 109 8 | |
| Totals | | 69 | 163 | 12 | 63 | 5 | 27 | 1 | 254 2 | 108.8 |

DISCUSSION OF RESULTS.

The total yields of tubers and the yields from the different grades of set are shown in Table 2. The amounts of ware, seed, and chats are shown in the same table. Taking Clone I it will be seen that the average yield per healthy plant was 3 lb. 6 oz (equivalent to $17\frac{1}{2}$ tons per statute acre); that strain (a) infected plants yielded 3% less than the healthy, while strain (b) infected plants out-yielded the healthy by 13%. The Up-to-Date stocks which were the sources of the infecting strains, and of which the sets were graded to correspond with those of Clone I, outyielded the healthy plants of Clone I by almost 9% in the case of strain (a) and by 3% in the case of strain (b) stock.

In the case of Clone II, the average yield per healthy plant was 3 lb. 10 oz ($18\frac{1}{2}$ tons per acre), strain (a) infected plants outyielded the healthy by 4%, while strain (b) infected plants produced 14% less than the healthy. It will be noted, however, that it was in the latter group that the primary leaf roll appeared, and examination of Table 2 shows that the reduced yield occurred amongst the plants of Grade D in which group the leaf roll was found. Actually, for ease in handling, the tubers of this grade had been dug and weighed in two sections, and this narrowed down the low yielding section to the last 27 plants of the drill which included those infected with leaf roll. Furthermore, the drop in yield in this section occurred exclusively in the ware size tubers, the weights of seed size tubers and chats from these 27 plants being greater than that of either healthy or strain (a) infected plants of the same clone. It seems evident, therefore, that the primary leaf roll effected a pronounced reduction in yield, and that there is no justification on the basis of these figures for assuming that any significant reduction in yield was caused by the (b) strain of virus X. The only other case of reduced yield, that of 3% in the (a) infected plants of Clone I, is counterbalanced by an increase of the same order in the corresponding plants of Clone II. Assuming that leaf roll was mainly responsible for the drop in yield in the strain (b) infected plants of Clone II, the general inference to be drawn from the results is that no significant reduction in yield is caused by the mild strains of X employed, but that there may even be an increase in yield due to the infection under certain conditions.

It is obvious, however, that yield trials of this type must be carried out over a number of seasons in order to compute the effects of varying weather conditions. In this particular experiment what promised to be a magnificent crop was marred by the abnormal rainfall, the average weight of tubers per plant was 3 lb. 8 oz., whereas the appearance of the crop in mid-summer justified an expectation of at least 5 lb. per plant, given normal weather conditions throughout the season. Furthermore, although the killing of maincrop plants by Blight in September or early October is a usual occurrence in this climate, in a season of average rainfall senescence would be well advanced at this period, and the effect of the disease on yield comparatively slight. In 1946, however, the continuous wet weather in August retarded senescence, so that the death of the plants in September due to Blight attack was definitely premature. It might reasonably be suggested that if the plants had matured normally the comparative yields of healthy and infected ones would have been different. In this connection, however, the yields obtained from the 1945

propagation plot (Table 1) are of interest. The sets used in this plot were the selected produce of pot plants and were all uncut and approximately 80—100 grms in weight. Throughout the season, the plants presented an appearance of uniform vigour and health, and owing to dry weather conditions in late summer Blight attack was comparatively mild. The average yield per plant was 5 lb., which is equivalent to almost 26 tons per statute acre. It will be seen that the strain (*a*) infected plants of each clone gave practically the same yield as the corresponding healthy plants, while the strain (*b*) infected plants outyielded the healthy, in one case by the substantial margin of 30%. Owing to the small numbers of plants involved it cannot be claimed that these figures are truly representative, but at least they suggest that in a season of average rainfall in this country the yielding capacity of Up-to-Date is not adversely affected by the presence of mild strains of X.

Whether or not the results of future experiments in different weather conditions continue to show the same trend, the fact emerges that plants infected with mild strains of X may sometimes outyield healthy plants of the same clone. So far, no confirmation of this tendency is to be found in the results of other investigators, but comparatively few trials have been carried out and, apart from differences in the strains of X employed, growth conditions vary at the different centres where such trials have been made. Scott's (10) experiment, although performed under conditions which probably approximated closely to those at Glasnevin, is not comparable with the present one, for he was concerned with the effect on cropping capacity of mosaic diseases in general, irrespective of the underlying viruses, his "negligible mottle" might have been due either to virus A or virus X, and his control plants, according to Smith and Markham (11), were probably carriers of latent X. The writers' experiment would be more or less equivalent to that of Smith and Markham (11), who also estimated the effect on yield of a mild strain of X, were it not for the fact that Smith and Markham inoculated their experimental plants in the field, whereas at Glasnevin the diseased plants were grown from infected sets. Experience here with a severe strain of X has shown that symptoms are invariably more severe following initial infection with the virus than at any subsequent stage of the host plant's growth; this effect is also apparent, although less obvious, in the case of milder strains of X. As it is possible, therefore, that yields would be affected differently according to the time of infection of the plants, the experiments at Glasnevin and Cambridge are not strictly comparable.

Schultz and Bonde (9) found consistent reductions in yield due to virus X infection in American varieties of potato. The disease tested was "common latent mosaic," which probably corresponds to some extent with the (*b*) strain of X used in the present work; but it is doubtful whether in general the virus strains underlying this disease are as mild as those used here. In the case of commercial Green Mountain, of which no healthy stock is available, Schultz and Bonde refer specifically to a weak strain of X which they tested against "common latent mosaic" in the same variety. The weak strain outyielded the other by 9%, which was similar to the difference in yield of healthy and common latent mosaic-infected Green Mountain Seedling. From this result one might infer that the weak strain probably had no significant effect on the yield of commercial Green Mountain.

Bald (3) demonstrated conclusively that under Australian conditions infection with different mixtures of X strains depresses the yields of Up-to-Date and other varieties of potato to an extent commensurate with the severity of the strains involved. He also observed that different clones of X-free Up-to-Date differed significantly in yielding capacity. On comparing the yields of X-free and X-infected Up-to-Date (4) he found in two experiments a reduction in yield due to the infection, although in only one case was the difference significant. In one small experiment, Bald (3) worked specifically with a very mild strain of X in a variety believed to be Great Scot, and found that the infected plants yielded 10% less than the healthy. Unfortunately growth conditions in this experiment were very unfavourable owing to excessive dryness, with the result that yields of both healthy and infected plants were exceedingly small.

While the results of other investigators demonstrate clearly that various strains of X reduce the cropping capacity of potatoes to a degree proportionate to the severity of the strain involved, the evidence relating specifically to the effects of mild strains of X is meagre. The logical assumption is, however, that such strains also depress yields, if only to a slight extent, the finding of an increase in yield due to infection is, therefore, difficult to explain. There is some evidence, however, that infection with virus X hastens maturity (3), (10), which presumably implies an earlier initiation of tuber formation, and Bald's (4) experiments show that the falling off in yield of infected plants is due to a reduction in the rate of tuberation in the final stages of growth. If it were a fact that infection with a mild strain of X induced earlier tuber formation it is conceivable that, in a climate in which crops are killed more or less prematurely by Blight or frost, such an effect might more than offset a slight reduction in the rate of tuberation in the senescent stage; thus, the yields of infected plants might be equal to, or greater than, those of healthy plants. It does not follow that a similar compensation would occur in the case of stronger strains of X, as the effect on time of tuber initiation might not be related to severity of strain in the same way as is the effect on rate of tuber formation.

The restricted incidence of leaf roll in the yield trial at Glasnevin serves to illustrate the serious effect on yield of the primary phase of this disease. It is clear that complete freedom from leaf roll must be aimed at in testing the comparative yields of healthy and X-infected plants, and herein lies one of the difficulties of conducting such trials on a large scale. Constant supervision is necessary, and the more extensive the plot the more danger there is that primary leaf roll may pass undetected, with a consequent unsuspected interference with the validity of the results.

SUMMARY.

A field trial was conducted to determine the effect of two different mild strains of virus X on the cropping capacity of Up-to-Date potato. Both strains were naturally occurring ones which are carried symptomlessly in Up-to-Date. Two clones of Up-to-Date were tested, from single tubers of which all the experimental plants were derived.

Weather conditions favoured luxuriant growth of the experimental plants during the first half of the season, but abnormal rain and lack of sunshine retarded senescence, and the plants were killed prematurely by Blight (*Phytophthora infestans*)

The average yield of tubers per healthy plant was 3 lb 8 oz, which is equivalent to 18 tons per statute acre. Strain (a) infected plants of Clone I yielded 3% less than the healthy, but those of Clone II outyielded the healthy by 4%. Strain (b) infected plants of Clone I outyielded the healthy by 13%, but those of Clone II yielded 14% less than the healthy. This reduction, however, was obviously due to an outbreak in mid-season of primary leaf roll, which was restricted to a number of adjoining plants in one particular drill. Neglecting the latter figure, the results in general indicated that no significant reduction in yield was caused by the latent strains of X employed, the tendency being rather towards an increase in yield due to the infection. The yields from a small propagation plot of the same Up-to-Date stocks in the previous season showed a similar trend.

The results are discussed and compared with those of other investigators.

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STUDIES ON UREIDES. PART 3

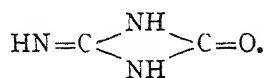
- (a) Constitution of Hallwachs' Acid.
 (b) A New Copolymer of Cyanamide and Cyanic Acid.

By

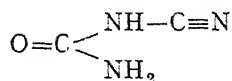
A. E. A. WERNER.

[Read March 25. Published separately July 9th, 1947].

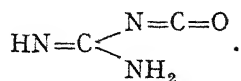
By the hydrolysis of dicyandiamide in dilute barium hydroxide solution Hallwachs (1) obtained in small yield a compound to which he gave the name amidodicyanic acid, and for which he proposed the cyclic structure



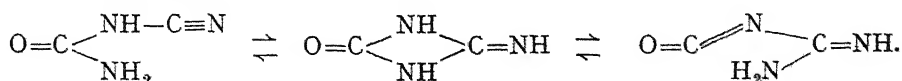
He also synthesised the potassium salt of the same substance in good yield directly from cyanamide and potassium cyanate. Baumann (2) showed that the compound could be hydrolysed on heating with dilute acids to give biuret; later Wunderlich (3) confirmed this observation and on the strength of it proposed the structural formula.



This so-called cyanurea structure has in the course of time gained general acceptance. Recently, however, Blair and Smith (4) offered for consideration a new formula, namely guanyl isocyanate,

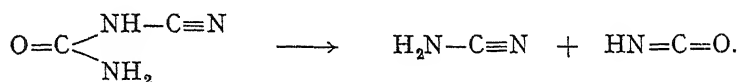


They were led to propose this formula in order to account for their observation that guanidine and urea are produced along with biuret during acid hydrolysis. They maintained that the cyanurea formula could not account for the formation of either urea or guanidine, and suggested that the chemistry of Hallwachs' acid was best explained by the assumption of tautomerism between a four-membered ring and two different chain structures as indicated below:—

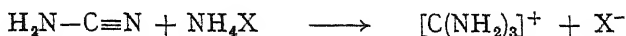


The new formula proposed by Blair and Smith (*loc. cit.*) lays itself open to the serious criticism that a compound possessing such a structure would not be expected to show acidic properties, but should instead show basic properties, since it contains a free guanidino group. Furthermore, since urea was only detected qualitatively, and guanidine could only be isolated in a very small yield, these substances must be regarded as by-products of the main hydrolysis, and therefore their formation hardly warrants the acceptance of a new formula. It is therefore proposed to retain the cyanurea formula for Hallwachs' acid, and to present experimental evidence in support of a mechanism of the hydrolysis which will account for the production of urea and guanidine as by-products of the main reaction

The necessary clue leading to the elucidation of the mechanism of hydrolysis, which provides a reasonable explanation of the formation of urea and guanidine was the discovery that cyanamide could be detected during the *initial* stages of the hydrolysis. It is suggested that the primary reaction in the hydrolysis is the dissociation of the cyanurea molecule into cyanamide and cyanic acid, ⁽¹⁾ thus —

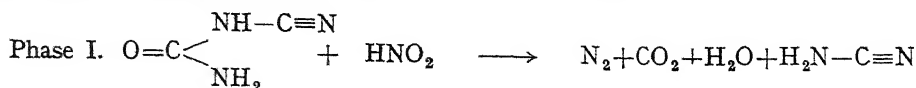


The cyanamide undergoes hydration to urea, which combines with cyanic acid to produce biuret. The yield of biuret is, however, well below the theoretical because much of the cyanic acid undergoes hydrolysis forming ammonium salts. Guanidine can be formed as the result of the union of some cyanamide with the ammonium salts, thus,

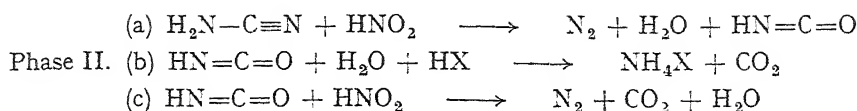


in accordance with the method for the preparation of guanidine salts described by Werner (E. A.) and Bell (5). This theory of the mechanism is in agreement with the fact that the hydrolysis of Hallwachs' acid in dilute acid solution is a slow process; the initial dissociation is the rate-determining reaction, and direct addition of the elements of water to the $-\text{CN}$ group does not occur. However biuret can be produced in good yield by the direct hydration of cyanurea by dissolving it in the cold in concentrated sulphuric acid, allowing to stand at room temperature, and then pouring into ice-cold water. (It is also shown that the same technique can be used to bring about the direct hydration of dicyandiamide to guanylurea [dicyandiamidine]). This direct hydration to give biuret affords further evidence in support of the cyanurea structure.

The theory outlined above was confirmed by an investigation of the action of nitrous acid on Hallwachs' acid. Cyanamide was detected qualitatively in the initial stages of the reaction, and the residual solution was found to contain ammonia. A quantitative determination of the ratio of the volumes of carbon dioxide and nitrogen evolved under different experimental conditions was found to be in agreement with the following theory of the course of the reaction:—



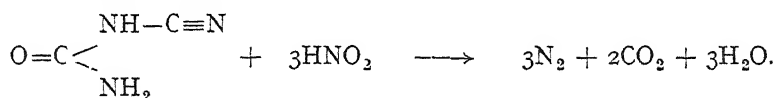
¹ Although Blair and Smith (*loc. cit.*) detected the formation of cyanic acid by its pungent odour, they failed to detect any cyanamide.



If the concentration of free mineral acid is high, reaction (b) will be favoured; whereas if the concentration of mineral acid is low and that of nitrous acid moderately high, reaction (c) will be favoured. Hence it follows that under experimental conditions so chosen that the concentration of mineral acid is high the overall reaction may be represented by the following equation:—



so that the volumes of carbon dioxide and nitrogen will be equal. On the other hand in the presence of an excess of nitrous acid an additional source of nitrogen is introduced so that the ratio of the volume of nitrogen to that of carbon dioxide will approach the theoretical ratio of 1.5 required by the following equation.—

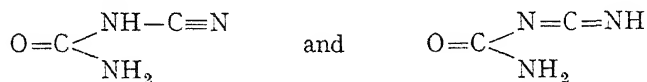


The above considerations are borne out by the experimental results collected in Table I, particularly the fact that in the presence of acetic acid and excess nitrous acid the ratio is very nearly equal to the theoretical required. On the other hand when hydrochloric acid is used to generate the nitrous acid, reaction (b) is never completely suppressed, and intermediate values for the ratio are obtained.

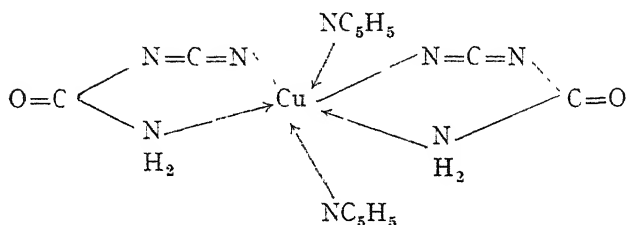
TABLE I. THE ACTION OF NITROUS ACID ON CYANUREA.

| Cyanurea millimols | Sodium Nitrite millimols | Acid added | Volume of Nitrogen c c. | Volume of Carbon Dioxide c c. | Ratio N_2 / CO_2 |
|-----------------------|--------------------------------|-------------------|-------------------------------|--|-------------------------------------|
| I | 2 | 2 c.c. NHCl | 17.0 | 13.3 | 1.28 |
| I | 2 | 3 c.c. NHCl | 29.9 | 21.4 | 1.12 |
| I | 2 | 1 c.c. 30% HCl | 26.8 | 26.0 | 1.03 |
| I | 3 | 1 c.c. 30% HAc | 13.8 | 9.4 | 1.47 |
| I | 4 | 1 c.c. 30% HAc | 16.5 | 11.2 | 1.48 |
| I | I | 3 c.c. NHCl | 10.7 | 10.95 | 0.99 |

A further point worthy of mention in connection with the structure of cyanurea is the fact that the phenomenon of tautomerism must play an important part in stabilising the molecule. Neglecting tautomerism in the acid amide part of the molecule there are still two possible tautomeric structures, namely,

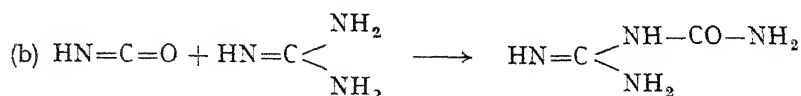


The unino tautomer is most probably the form from which the covalent heavy metal salts are derived, and this idea receives additional support from the preparation of a new deep blue copper pyridine salt corresponding to the formula, $(\text{C}_2\text{H}_2\text{N}_3\text{O})_2\text{Cu} \cdot 2\text{C}_5\text{H}_5\text{N}$, and having the probable chelate structure shown below—



The second part of the present communication is concerned with an interesting substance which is isomeric with Hallwachs' acid, but yet possesses entirely different chemical and physical properties. It seems probable that this substance was originally obtained by Wunderlich (*loc. cit.*) as the result of the action of heat on guanolin (carbethoxyguanidine), and later by Soll and Stutzer (6), who make a passing reference to the formation during the thermal decomposition of guanylurea of a white insoluble material, the analysis of which approximated to that required for $\text{C}_2\text{H}_3\text{N}_3\text{O}$. However neither the chemical nor physical properties of this substance are described in either of the above mentioned papers, Wunderlich (*loc. cit.*) merely stating that it is entirely different from the isomeric compound obtained by Hallwachs (*loc. cit.*).

It is now shown that when guanylurea hydrochloride is heated to 175°C . it decomposes completely in twenty minutes giving ammonium chloride and this new substance in 90% yield together with a small amount of ammeline. The same substance is also obtained by the action of heat on an equimolecular mixture of guanidine carbonate and urethane. This latter reaction was originally described by Bamberger (7) as a method for the preparation of guanylurea, which was supposed to be formed as the result of the following sequence of reactions—



In a repetition of Bamberger's work attempts to isolate any guanylurea *via* its characteristic red copper salt were unsuccessful this is, however, not altogether

surprising, since Werner (E. A.) (8) has shown that urethane only dissociates to a very small extent even at its boiling point (187°C). Therefore the formation of guanylurea according to the above series of reactions must be regarded as extremely unlikely. As shown in the experimental part the reaction between urethane and guanidine carbonate leads instead to the intermediate formation of guanolin:—



Guanolin can be isolated from the reaction mixture if the temperature does not rise above 110°C, but at the temperature of 150°C used by Bamberger (*loc. cit.*) the guanolin decomposes as rapidly as it is formed with the evolution of ethyl alcohol vapour, and the new compound is formed. This reaction mechanism receives further support from the observation that the ammonia evolution occurs at a lower temperature (70°C) than the evolution of ethyl alcohol vapour, which only starts at 120°C., i.e. above the decomposition point of guanolin.

Attention was also directed to the chemical reaction resulting from the action of heat on an equimolecular mixture of urea and guanidine carbonate. This reaction was first investigated by Smolka and Friedreich (9), who claimed that it led to the formation of diguanylbiuret (duminotetruret) of the formula, $\text{H}_2\text{N}-\text{C}(\text{NH})-\text{NH}-\text{CO}-\text{NH}-\text{CO}-\text{NH}-\text{C}(\text{NH})\text{NH}_2$. There are however two cogent reasons for doubting the accuracy of the claim put forward by these workers. Firstly the properties ascribed by Smolka and Friedreich (*loc. cit.*) to the supposed diguanylbiuret are exactly those possessed by the new compound (*vide infra*), and secondly convincing evidence has been recently presented by Werner and Gray (10) to show that the existence of the compound tetruret itself must be regarded as extremely doubtful. In point of fact it is now shown that when equimolecular amounts of urea and guanidine carbonate are heated to about 130°C. the new compound is obtained in almost quantitative yield.

Since the properties of this new substance have not been previously described, certain of its more important features are detailed below in order to provide evidence in support of a suggested structure for the substance:—

(a) The compound does not show any true melting point; on heating to about 450°C. decomposition takes place with the evolution of ammonia and cyanic acid vapour, which form a sublimate of ammonium cyanate in a cooled receiver, some melamine is deposited on the walls of the test-tube and a golden yellow residue of melon remains. The formation of these decomposition products may be explained on the assumption that the compound breaks up initially into cyanamide and cyanic acid. The cyanamide polymerises to the trimeric melamine, which at the higher temperature loses ammonia to form melam, which in turn is finally converted into the stable melon.

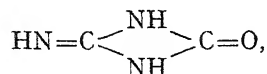
(b) The compound is insoluble in water and the usual organic solvents, but dissolves to a small extent in formamide on warming.

(c) The compound shows amphoteric properties, dissolving in acids to give salts of the general formula $\text{C}_2\text{H}_3\text{N}_3\text{O} \cdot \text{HX}$, and in alkalis to give salts of the general formula $\text{M} \cdot \text{C}_2\text{H}_2\text{H}_3\text{N}_3\text{O}$, which are decomposed to the parent substance by carbon

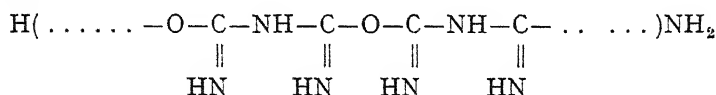
dioxide. It does *not* dissolve in concentrated ammonium hydroxide. A silver salt was also prepared and found on analysis to have the empirical formula $\text{AgC}_2\text{H}_2\text{N}_3\text{O}$.

(d) The compound is chemically rather inert; it resists the oxidative action of alkaline hypobromite, and its resistance to hydrolysis is exemplified by the fact that its solution in N potassium hydroxide can be refluxed for ten hours without undergoing appreciable decomposition.

The above chemical and physical properties are definitely not in agreement with the idea that this new compound possesses the cyclic structure

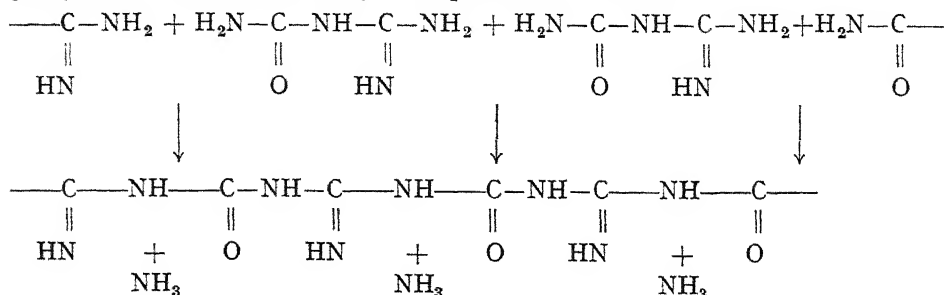


which was proposed by Wunderlich (*loc. cit.*). They suggest rather that the compound has a high molecular weight, and, in accordance with the theoretical considerations established by Carothers (11) which indicate the improbability of the formation in bifunctional condensation reactions of rings containing more than eight atoms, it seems that an open chain structure is the most likely one. Since the compound contains cyanic acid and cyanamide in equimolecular proportions it is suggested that it is best represented as a macromolecular *linear copolymer* of cyanic acid and cyanamide, namely:—



The exact nature of the terminal groups is unknown, but, bearing in mind the experimental conditions under which the copolymer is produced, it is probable that the addition of a molecule of ammonia occurs, giving an $-\text{OH}$ group at one and an $-\text{NH}_2$ group at the other end of the chain, as indicated in the above formula. A similar open chain structure has also been advanced for cyamelide, the low temperature polymer of cyanic acid. (*Vide* Sidgwick's Organic Chemistry of Nitrogen, 2nd edition, p. 323.).

The actual formation of the copolymer takes place as the result of a polycondensation involving the *intermolecular* elimination of ammonia between the guanido group of one molecule and the carbamino group of a second molecule of guanylurea. The reaction may be represented as follows:—



The alternative formation of the copolymer from guanolin may be similarly represented as a polycondensation reaction involving the intermolecular elimination of ethyl alcohol between the guanido group of one molecule and the carbethoxy

group of a second molecule of guanolin. It is of incidental interest to contrast these reactions with the thermal decompositions of the urea analogues, ethyl allophanate and biuret. The former decomposes on heating above its melting point ($190^{\circ}\text{C}.$) forming cyanic acid, which undergoes polymerisation to the cyclic cyanuric acid, while Werner (E. A.) (12) has shown that biuret decomposes in a complex manner leading to the formation of cyanuric acid and ammelide, which also has the stable 1, 3, 5-triazine ring structure. This same ring system is also present in melamine, the trimeric polymer of cyanamide, and in ammeline, which may be regarded as a condensation product of cyanic acid and cyanamide in the ratio of 1 : 2. It is thus clear that the new copolymer described in the present communication is of particular interest, because it represents a unique example of a linear condensation product of cyanamide and cyanic acid. Owing to the insolubility of the substance in appropriate solvents it is unfortunately impossible to determine the number of repeating units in the chain, but its physical character would indicate that the number of such units is quite large, as is known to be the case with the polyoxymethylenes, in which Staudinger and his associates (13) have shown that the chain contains from forty to one hundred units.

EXPERIMENTAL.

Hydrolysis of Cyanurea. A solution of cyanurea (4.25 g.) in $N/2$ sulphuric acid (100 c.c.) was heated slowly on a water-bath; as the temperature rose test portions of the solution were withdrawn and examined for the presence of cyanamide. At $70^{\circ}\text{C}.$ the presence of cyanamide was detected by the characteristic pale yellow precipitate of silver cyanamide insoluble in ammonium hydroxide solution. The solution was refluxed at $80^{\circ}\text{C}.$ for 90 minutes, during which the characteristic odour of cyanic acid was noticeable. The solution was allowed to cool and was titrated with N sodium hydroxide. Volume required was 30 c.c. Hence the amount of ammonia produced as a result of the hydrolysis of cyanic acid is equivalent to 40 c.c. of $N/2$ sodium hydroxide corresponding to 40% of the theoretical.

The above experiment was repeated, and the resulting solution was rendered alkaline by the addition of a small volume of concentrated sodium hydroxide solution, and aerated until all the ammonia had been expelled. The solution was then concentrated to a small bulk under reduced pressure. The crystalline material which separated was filtered and recrystallised from ethyl alcohol. M.P. $188^{\circ}\text{C}.$ not depressed by mixing with an authentic specimen of biuret. Weight = 1.05 g. equivalent to 20% of the theoretical. Guanidine was found to be present in the filtrate in small amount, being identified by means of its picrate. M.P. $310^{\circ}\text{C}.$

Action of Nitrous Acid on Cyanurea. All experiments were carried out in a Lunge nitrometer filled with mercury, and the following technique was employed. One millimolecular proportion (0.085 g.) of cyanurea and the requisite quantity of recrystallised sodium nitrite dissolved in distilled water (2 c.c.) were introduced into the nitrometer. The nitrometer cup was rinsed out with distilled water (1 c.c.), the required amount of acid was introduced into the nitrometer, and the total volume of the solution was adjusted to a volume of 6 c.c. by adding distilled water if required. Except in those experiments in which acetic acid was used the evolution

of gas was fairly brisk. The experiment was allowed to continue for 18 hours. The total volume of gas in the nitrometer was noted, and the gas was analysed for (i) carbon dioxide by noting the contraction in volume resulting upon the addition of concentrated solution of sodium hydroxide, and (ii) nitric oxide by noting the contraction in volume after the addition of a strong solution of potassium permanganate. The residual gas in the nitrometer was assumed to be nitrogen. All gas volumes were reduced to N.T.P., and a suitable allowance was made for the volume of carbon dioxide which remains dissolved in the aqueous solution.

Preparation of a Copper Pyridine salt from Cyanurea. To an aqueous solution of the potassium salt (1.14 g) were added pyridine (3 c.c.) and a 5% solution of cupric sulphate (25 c.c.). On standing for a few hours deep blue acicular crystals gradually separated out of the solution. The crystals were dried in a desiccator over sulphuric acid. On ignition 0.1228 g. gave 0.0248 g. CuO, equivalent to Cu=16.10 per cent. $(C_2H_2N_3O)_2Cu.2C_5H_5N$ requires Cu=16.19 per cent.

Direct Hydrolysis of Cyanurea and Dicyandiamide. (i). To concentrated sulphuric acid (20 c.c.) cooled to $-10^\circ C.$ was added in small portions cyanurea (5 g), the rate of addition being such that the temperature did not rise above $0^\circ C.$ The cyanurea dissolved without effervescence giving a clear solution. A test portion of the solution was found to give the biuret reaction when poured into water and tested in the usual manner. The main solution was allowed to stand at room temperature for one hour, and was then poured with vigorous stirring on to crushed ice (ca. 100 c.c.). The resulting solution was treated with solid barium carbonate until neutral, the barium sulphate was removed, and the filtrate was evaporated to a small bulk under reduced pressure. The crystalline material which separated was filtered off and recrystallised from ethyl alcohol. Weight=4.5 g. corresponding to 75 per cent. of the theoretical. M.P. $190^\circ C.$ not depressed by admixture with an authentic specimen of biuret.

(ii). The above technique was repeated using dicyandiamide (5 g). Test portions of the sulphuric acid solution gave positive reactions showing the presence of guanylurea when tested with copper sulphate (characteristic salmon pink precipitate) and with nickel chloride (characteristic yellow precipitate). After neutralising the main solution with barium carbonate the guanylurea was isolated via its copper salt, which was decomposed by hydrogen sulphide. The precipitated copper sulphide was removed, and the filtrate was evaporated to crystallisation point under reduced pressure. Weight of crystalline material obtained was 4.8 g., equivalent to 76 per cent. of the theoretical. After recrystallisation from ethyl alcohol M.P. $104^\circ C.$

Action of Heat on Guanylurea Hydrochloride. Finely-powdered, guanylurea hydrochloride (10 g.) was spread in a thin layer in a conical flask heated in an oil-bath. The mass started to melt at $170^\circ C.$, a strongly exothermic reaction occurred causing sharp rise in temperature, and the fused mass resolidified as the infusible reaction product formed; after 20 minutes heating the reaction was completed. The solid residue was extracted with boiling water to remove ammonium chloride, and then dried. Weight=5.5 g. corresponding to 87 per cent. of the theoretical. The hot filtrate deposited a small amount of microcrystalline solid on cooling, which was identified as ammelide. Found N=44.21 per cent.; $C_3H_4N_4O_2$ requires N=43.75 per cent. The main solid was dissolved in N sodium hydroxide (70 c.c.), filtered from a small amount of insoluble matter, and precipitated by a current of carbon

dioxide gas as a pure white microcrystalline powder. This was filtered off, washed free of sodium bicarbonate, and analysed. Found $C=28.30$, $H=3.96$; $N=48.98$ per cent. $C_2H_3N_3O$ requires $C=28.24$, $H=3.53$, $N=49.42$ per cent. A silver derivative was prepared by adding N silver nitrate solution (25 c.c.) to a solution of the compound (2.12 g.) in N potassium hydroxide (30 c.c.). The white precipitate formed was filtered off and dried. Weight = 3.8 g. Found $Ag=55.81$ per cent. $AgC_2H_2N_3O$ requires $Ag=56.25$ per cent.

Action of heat on an equimolecular mixture of urethane and guanidine carbonate.

(i). An intimate mixture of urethane (9 g.) and guanidine carbonate (9 g.) was heated in a conical flask immersed in an oil-bath and fitted with a delivery tube. The evolution of ammonia gas commenced at $70^\circ C$, and ethyl alcohol started to distil over at $120^\circ C$, at $130^\circ C$. a white sublimate collected in the delivery tube, which proved on examination to be ammonium carbonate. The temperature was maintained at $140^\circ C$ for about 60 minutes, by which time all action had ceased. The solid in the flask was extracted with water, in which guanylurea is readily soluble, and filtered. The insoluble residue was dissolved in a dilute solution of sodium hydroxide, and precipitated by carbon dioxide as a white powder, which was filtered off and dried. Weight = 6.75 g., corresponding to 75 per cent. of the theoretical. Found $N=49.35$ per cent.; $C_2H_3N_3O$ requires $N=49.42$ per cent. The filtrate failed to give the characteristic reddish precipitate of guanylurea upon the addition of copper sulphate and sodium hydroxide solutions.

(ii). A finely-powdered mixture of urethane (4.5 g.) and guanidine carbonate (4.5 g.) was heated as in the previous experiment, but the temperature was not allowed to exceed $110^\circ C$. The heating was continued until the evolution of ammonia gas had ceased. The dry residue was recrystallised from ethyl alcohol. Weight = 5.6 g., corresponding to 86 per cent. of the theoretical. After drying over sulphuric acid in a vacuum desiccator the M.P. was $118^\circ C$. Nencki (14) gives $114^\circ C$., and Basterfield and Paynter (15) give $120^\circ C$ as the M.P. of guanolin. The identity with guanolin was confirmed by the preparation of the sparingly soluble nitrate. M.P. $181^\circ C$. Pinck and Blair (16) give M.P. $182-183^\circ C$.

Action of heat on an equimolecular mixture of urea and guanidine carbonate.

A mixture of urea (6 g.) and guanidine carbonate (9 g.) was heated as in the previous experiments. At $50^\circ C$ the evolution of ammonia gas commenced, and the mass began to fuse at $110^\circ C$. The temperature was maintained constant at $130^\circ C$. while a brisk effervescence occurred and a sublimate of ammonia carbonate collected in the cold receiver. After one hour the evolution of ammonia gas ceased, and the heating was discontinued. The solid residue was dissolved in dilute sodium hydroxide solution, precipitated by a current of carbon dioxide gas, filtered off, and dried. Weight = 8.0 g., corresponding to 94 per cent. of the theoretical. Found $N=49.65$ per cent. $C_2H_3N_3O$ requires $N=49.42$ per cent. Silver salt prepared and analysed. Found $Ag=57.00$ per cent. $AgC_2H_2N_3O$ requires $Ag=56.25$ per cent.

Formation of Salts with Acids. (i) Nitrate. The copolymer dissolved in concentrated nitric acid on gentle warming, and after the addition of a small amount (ca. 10%) of water the nitrate separated on cooling in the form of small rectangular prisms. On heating the compound decomposed with the evolution of brown fumes at $202^\circ C$. without melting. On titration the salt (0.192 g.) required for neutralisation 13.26 c.c. of $N/10$ sodium hydroxide. $C_2H_3N_3O.HNO_3$ would require 13.0 c.c.

(ii) *Sulphate*. The copolymer is dissolved in cold concentrated sulphuric acid, and an equal volume of ice-cold water is added. The clear solution slowly deposited the sulphate in the form of spheroidal granules. On titration the salt (0.158 g) required for neutralisation 8.00 c.c. of $N/10$ sodium hydroxide, corresponding to the formula $(C_2H_3N_3O)_2 \cdot H_2SO_4 \cdot 7H_2O$. This formula was confirmed by drying the salt in a vacuum desiccator. Loss in weight = 31.5 per cent, the heptahydrate would require a loss in weight = 31.99 per cent.

(iii) *Hydrochloride*. The copolymer dissolved on warming in concentrated hydrochloric acid diluted with an equal volume of water, and on cooling the hydrochloride salt separated as a flocculent microcrystalline precipitate. On titration the salt (0.079 g.) required for neutralisation 6.80 c.c. of $N/10$ sodium hydroxide. $C_2H_3N_3O \cdot HCl$ would require 6.50 c.c.

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STUDY OF THE POLYMERISATION OF CYANIC ACID, WITH SPECIAL
REFERENCE TO DICYANIC ACID

By

A. E. A. WERNER AND J. GRAY

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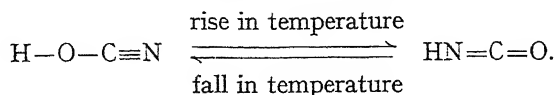
SENIER and Walsh (1) showed that when liquid cyanic acid underwent polymerisation at 0°C. the resultant white solid consisted of a mixture of two polymers, namely cyanuric acid and cyamelide, the former being present to the extent of 70 per cent. Later Werner (E. A.) and Fearon (2) studied the polymerisation of cyanic acid at temperatures ranging from 0°C. to 20°C., and found that the proportion of cyamelide in the polymer mixture was greater the lower the temperature. This observation has now been extended to lower temperatures, and, as shown in Table I, the yield of cyamelide can be increased to almost 70 per cent. by carrying out the polymerisation at -20°C.

TABLE I. THE POLYMERISATION OF CYANIC ACID.

| Temperature of Polymerisation | Weight of Polymeride | Cyanuric Acid Found | Percentage Composition of Polymeride | |
|-------------------------------------|----------------------------|---------------------------|---|---------------|
| | | | Cyamelide | Cyanuric Acid |
| 0°C. | 0.541 g. | 0.218 g. | 59.6 | 40.4 |
| | 0.532 g. | 0.219 g. | 58.8 | 41.2 |
| -10°C. | 0.562 g. | 0.213 g. | 62.1 | 37.9 |
| | 0.550 g. | 0.202 g. | 63.2 | 36.8 |
| -15°C. | 0.609 g. | 0.206 g. | 66.1 | 33.9 |
| | 0.652 g. | 0.223 g. | 65.8 | 34.2 |
| -20°C. | 0.448 g. | 0.135 g. | 69.8 | 30.2 |

Cyamelide is also produced in good yield when a well-cooled suspension of cyanic acid is acidified with dilute hydrochloric acid.

The results tabulated above provide further support for the theory of polymerisation of cyanic acid advanced by Werner (E. A.) (3), who postulated that cyanic acid exists as a keto-enol equilibrium mixture.—



Cyamelide is formed by the polymerisation of the low temperature enol form, whereas cyanuric acid results from polymerisation of the keto form. Hence the relative proportions of the two polymerides formed reveal the composition of the equilibrium mixture at any particular temperature.

Owing to the formal analogy between the chemical formula of cyanic acid, $\text{HN}=\text{C}=\text{O} \rightleftharpoons \text{HO}-\text{C}\equiv\text{N}$, and that of cyanamide, $\text{HN}=\text{C}=\text{NH} \rightleftharpoons \text{H}_2\text{N}-\text{C}\equiv\text{N}$, attempts have been made to prepare a dimeric form of cyanic acid which would bear the same relationship to the dimeric dicyandiamide as the trimeric cyanuric acid does to the trimeric melamine. Ponsgen (4) originally claimed to have obtained a 'dicyanic acid', but it was shown by Hallwachs (5) that the substance was merely impure cyanuric acid. Schmidt (6) also claimed to have obtained a small amount of 'dicyanic acid' by the action of excess phosgene on triuret, but the evidence presented by Schmidt (*loc. cit.*) was very inadequate, and he relied chiefly on the isolation of a silver salt, the analysis of which approximated to that required by the formula $\text{Ag}_2\text{C}_2\text{HN}_2\text{O}_2$.

There were no further references in the literature to the existence of 'dicyanic acid' until Davis and Blanchard (7) produced evidence purporting to show that cyanic acid in aqueous and in alcoholic solution dimerises (in part at least) to form a 'dicyanic acid' according to the following equation:—

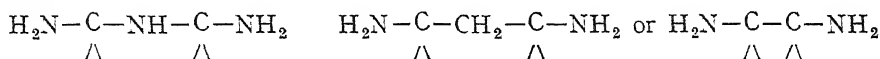


Davis and Blanchard (*loc. cit.*) did not succeed in isolating this 'dicyanic acid', but claimed to recognise its existence in solution by the fact that the well-known biuret reaction was given by solutions which apparently did not contain any actual biuret, since they were unable to isolate biuret from the solid residue remaining after evaporating these solutions to dryness. The pseudo-biuret reaction was assumed to indicate the presence of 'dicyanic acid', which they failed to isolate because it was completely hydrolysed during the evaporation.

In view of the rather unsatisfactory nature of the experimental evidence adduced in support of the existence of 'dicyanic acid', to which moreover Davis and Blanchard (*loc. cit.*) have assigned an important role as an intermediate in the formation of allophanates from alcohols and cyanic acid, the present authors have carefully repeated the experiments described by Davis and Blanchard (*loc. cit.*). Our results, however, do not substantiate their claim to have demonstrated the existence of the postulated 'dicyanic acid' in solution. The positive biuret reaction given by an aqueous solution of cyanic acid, prepared by acidifying sodium or potassium cyanate with the required amount of acetic or hydrochloric acids, was in all cases traced to the presence of biuret, which could in every case be isolated

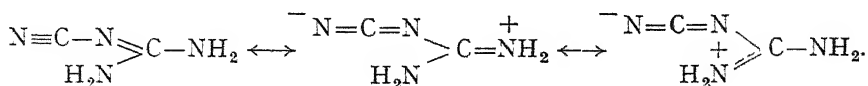
from the solution upon evaporation. Davis and Blanchard (*loc cit.*) also claimed that 'dicyanic acid' was formed in the decomposition of both nitrourea and nitrobiuret, but we have found that the positive biuret reaction which developed when an aqueous solution of either nitrourea or nitrobiuret was heated until effervescence commenced was due to the actual presence of biuret. In no case was there ever any indication that a positive biuret reaction was due to any substance other than biuret itself. The formation of biuret under the above experimental conditions is, moreover, to be expected, and its formation is readily explained by the mechanism suggested by Werner (E. A.) and Fearon (*loc cit.*).

It must also be pointed out that it is extremely doubtful if a substance having the structure postulated for 'dicyanic acid' would be expected to give a positive biuret reaction. Extensive investigations by Schiff (8) on the constitution of the complex responsible for the positive biuret reaction have shown that only those substances give a positive reaction which possess one of the following groupings in the molecule:—



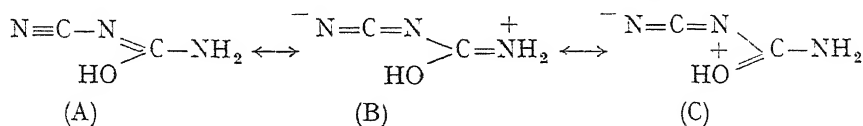
Typical representatives of the three classes are biuret itself, malonamide, and oxamide. The capacity to form a five- or six-membered chelate ring is an essential criterion for a positive biuret reaction. It is clear that 'dicyanic acid' does not conform to the necessary requirements. Furthermore it has been shown by Jesserer (9) that a refinement of the biuret reaction is possible which permits one to distinguish between the colour reaction obtained with biuret and its immediate derivatives and that given by the compounds belonging to the malonamide and the oxamide classes. The violet copper biuret complex consists of a red and a blue component; the former is produced alone upon the addition of the first few drops of a dilute copper sulphate solution, giving a characteristic *rose-red* colour, which only changes to the usual *violet* colour upon the addition of a further quantity of copper sulphate solution. In all our experiments this characteristic colour sequence was observed when testing for biuret. This affords additional proof that the biuret reaction observed in our experiments was due to the presence of biuret, and not 'dicyanic acid'.

It is finally of interest to discuss possible reasons why 'dicyanic acid' is not a stable substance, particularly in view of the established existence of *dicyandiamide*, the dimer of cyanamide, and Hallwachs' acid or *cyanurea*, a codimer of cyanic acid and cyanamide. The cyanguanidine structure for dicyandiamide has recently been definitely established as the result of an X-ray crystallographic analysis by Hughes (10), who showed that it exists as a resonance hybrid of the structures:—

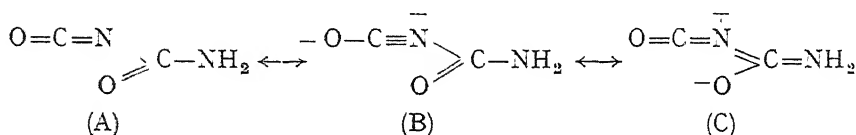


Since all three structures contribute almost equally to the resonance hybrid, the resonance energy of the dicyandiamide molecule is large, and undoubtedly is responsible for the relative stability of dicyandiamide. The cyanurea structure for Hallwachs' acid has not yet been investigated by the X-ray crystallographic technique, but there is a considerable amount of chemical evidence (as shown in the preceding

communication) in support of such a structure for Hallwachs' acid. On the basis of such a cyanurea structure the positions of the atoms would correspond to a molecule, which may be represented as a resonance hybrid, involving mainly the structures (A) and (B) but with a smaller contribution from the structure (C).



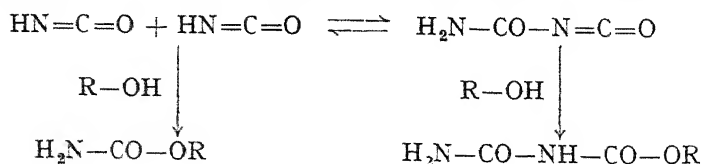
In this case the resonance is not so complete, the resonance energy is consequently smaller, and the compound will be less stable than dicyandiamide (as is actually the case) but still sufficiently stable to be easily isolated and withstand heating in aqueous solution up to a temperature of 80°C. However in the case of 'dicyanic acid' a similar type of resonance is impossible, the contribution of the postulated components (B) and (C) of the possible resonance system shown below —



will be almost negligible—hence there will be no gain in stability due to resonance energy, and 'dicyanic acid' may therefore be expected to be less stable than either dicyandiamide or Hallwachs' acid.

Another point of interest, which is apposite to the present discussion, is the existence of thiodicyanic acid, $\text{H}_2\text{N}-\text{CO}-\text{N}=\text{C}=\text{S}$, obtained recently by Birckenbach (11) in good yield by the condensation in ethereal solution at 0°C. of equimolecular amounts of cyanic and thiocyanic acids. This compound can be heated up to 105°C. before undergoing decomposition, but is *instantly hydrolysed in the presence of traces of moisture*. Now it is a well-known fact that the thio derivatives of carbanic acid are more stable than their oxygen analogues, and therefore the fact that thiodicyanic acid is so readily hydrolysed by traces of water is strong evidence against the possible existence of 'dicyanic acid' under the experimental conditions described by Davis and Blanchard (*loc. cit.*), and until further evidence is forthcoming the existence of 'dicyanic acid' must be regarded as extremely doubtful.

In view of the probable non-existence of 'dicyanic acid' it is now necessary to reconsider the claim advanced by Davis and Blanchard (*loc. cit.*) that when an aqueous solution of cyanic acid reacts with alcohols not only carbamates but also allophanates are formed, both however in small yields. The formation of the allophanates is ascribed by these authors to the presence of 'dicyanic acid' as an intermediate, and the series of reactions was represented as follows:—



We have repeated the experiments of Davis and Blanchard (*loc cit*), but were only able to isolate ethyl allophanate in very small yield when the reaction was carried out in 50 per cent. alcohol water mixture, and hydrochloric acid was present in slight excess. We found, moreover, that, if an excess of hydrochloric acid is avoided, the sole product of the reaction, in either absolute alcohol (which ought to be a better medium for 'dicyanic acid') or a 50 per cent. alcohol water mixture, was the carbamate. Ethyl allophanate was only produced in a reasonable yield when the reaction was carried out in absolute alcohol and hydrochloric acid was present in excess. These results are in agreement with the general mechanism presented by the authors in a previous communication (12), in which they showed that cyanic acid could combine with carbamates only in the presence of hydrochloric acid. It thus follows that the formation of allophanates is due to the addition of cyanic acid to the carbamate molecule, and does not proceed as the result of the union of an alcohol with the hypothetical 'dicyanic acid'.

EXPERIMENTAL.

Polymerisation of cyanic acid at low temperatures Samples of liquid cyanic acid, prepared by the thermal decomposition of anhydrous cyanuric acid at dull red heat, were confined in thin layers in narrow thin-walled tubes immersed in baths maintained at 0°C. (ice and water), -10°C. (ice and salt), -15°C and -20°C. (ice and calcium chloride in suitable proportions), respectively. This technique ensures a fairly accurate control of the temperature, and by efficient dissipation of the heat of polymerisation reduces to a minimum any tendency for the temperature to rise. When polymerisation was completed, a weighed quantity of the polymeride was extracted with hot water, and, after removal of the insoluble cyamelide, the cyanuric acid in solution was determined by titration with *N*/10 sodium hydroxide using phenolphthalein as indicator.

Cyanic acid in absolute ethyl alcohol solution. (a) Sodium cyanate (6.5 g.) was added in portions to 2*N* solution of hydrochloric acid in absolute ethyl alcohol (50 c.c.) maintained at about 10°C. The reaction mixture was left overnight, and then filtered to remove the insoluble sodium chloride. The alcoholic filtrate was concentrated in a vacuum desiccator over sulphuric acid, and the crystalline material which collected was dried and weighed. Weight=5.00 g., corresponding to 60 per cent. of the theoretical. M.P. 48°C., not depressed by admixture with an authentic specimen of ethyl carbamate. (b) Sodium cyanate (6.5 g.) was added in portions to a solution of hydrochloric acid in ethyl alcohol, and simultaneously a current of dry hydrochloric acid gas was passed, while the temperature was kept at about 10°C. by external cooling. After all the sodium cyanate had been added the reaction mixture was left overnight, and then filtered. The dried solid residue was extracted with boiling benzene, and yielded ethyl allophanate, M.P. 193°C., not depressed by admixture with an authentic specimen. Weight 4.2 g., corresponding to 64 per cent. of the theoretical. On concentration *in vacuo* the alcoholic filtrate yielded ethyl carbamate. M.P. 48°C. Weight=1.5 g.

Cyanic acid in aqueous alcoholic solution. Ethyl alcohol (30 c.c.) was added to a solution of potassium cyanate (16.2 g.) in distilled water (30 c.c.). The solution

was acidified with hydrochloric acid, allowed to stand at room temperature one hour, and then evaporated to dryness. Extraction of the solid residue (Wt = 20 g) with ether gave urethane M.P. 48°C. Weight = 0.40 g. corresponding to 2.3% of the theoretical. Extraction with boiling benzene yielded ethyl allophanate. M.P. 192°C. Weight 0.24 g. corresponding to 1.8% of the theoretical. The solid residue contained ammonium chloride. Wt = 5.5 g, corresponding to 50 per cent. of the theoretical derived from the hydrolysis of cyanic acid.

Formation of biuret in aqueous solutions of cyanic acid (a) Glacial acetic acid (6 c.c.) was added slowly to a well-stirred solution of potassium cyanate (8.1 g.) in water (20 c.c.). After neutralisation with sodium carbonate the solution gave the characteristic biuret reaction. The solution was evaporated to dryness on a water-bath, and the residue also gave a positive biuret reaction. This residue was extracted with a hot solution of potassium hydroxide to dissolve out the biuret. The alcoholic solution was acidified with hydrochloric acid, and after removal of the precipitated potassium chloride the filtrate gave a strong positive biuret reaction, and yielded a residue of impure biuret. Recrystallised from alcohol. M.P. 188°C. not depressed by admixture with an authentic specimen of biuret. (b) Concentrated hydrochloric acid (10 c.c.) was slowly added to a well-cooled (–10°C.) suspension of sodium cyanate (6.5 g) in distilled water (50 c.c.) A fine precipitate formed, and a strong odour of cyanic acid developed. After standing for one hour the solution was filtered. The residue proved to be cyamelide admixed with cyanuric acid, identified by means of its characteristic violet copper ammonium salt, and the filtrate gave a strong positive biuret reaction. On evaporating the filtrate to dryness and subsequent extraction with alcoholic potassium hydroxide biuret was isolated and identified by a mixed M.P. with an authentic specimen of biuret.

Decomposition of nitrourea in aqueous solution. A solution of nitrourea (2.0 g) in water (20 c.c.) was slowly evaporated to dryness on a water-bath during which effervescence occurred. The residue gave a positive biuret reaction, and a sample of biuret was isolated. M.P. 188°C. not depressed by admixture with an authentic specimen of biuret.

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No. 24.

NOTES ON THE ACIDITY, CHLORIDE CONTENT, AND OTHER CHEMICAL FEATURES OF SOME IRISH FRESH WATERS.

By

D. A. WEBB, Ph.D.

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IRELAND is a country rich in rivers and lakes, but comparatively little is known of the chemistry of its fresh waters. The observations in this field that are here reported have been made from the standpoint of the hydrobiologist. No complete analyses are presented, but attention has been focussed on certain constituents that are likely to be of biological importance. Some of the findings possess, however, a certain geochemical interest.

Part of the expenses incurred in these investigations were defrayed by a grant from the Society for this purpose.

pH.

Of all the chemical factors that govern the distribution of fresh-water organisms pH is probably the most important. It is well known, for example, that the majority of animals with a calcareous exoskeleton are unable to tolerate strongly acid waters; while in the plant kingdom a large number of aquatic, as of terrestrial species, are restricted to a certain range of pH in their environment.

Many fresh waters, especially those that are neutral or slightly acid, possess such a low buffer capacity that certain precautions must be observed if accurate determinations of pH are to be obtained. The determinations reported in this paper were all made on the spot, so that errors arising from change of acidity during transport or storage were eliminated. But all colorimetric determinations of weakly buffered solutions are subject to the possibility of error arising from the change of pH produced by the addition of the indicator itself, which, being the salt of a weak acid, may in an unfavourable case exert a greater influence on the final value of the pH than do all the original constituents of the water. Although this danger has several times been pointed out, the fact that figures are often published which appear to rest on a single colorimetric reading shows that it is not as widely appreciated as it should be.

There are several ways in which the disturbing influence of the indicator on the pH may be either discounted or eliminated. That which I have adopted is very simple, and is based on a method described by Clark (1920). Several distinct solutions of each indicator are made up, one with the normal quantity of alkali added (sufficient

for half neutralization), and the others with slightly differing quantities. The pH of each of these indicator solutions is then determined. This may be most conveniently done by using a Duboscq colorimeter to match a very small depth of the indicator solution against either a deeper layer of the indicator in a buffer solution of known pH or else a standard glass of a Lovibond Tintometer. In this way four solutions of bromothymol blue, for example, will be made up, with their pH at 6.2, 6.5, 6.8, and 7.2. When a similar series has been made up for the other indicators in use it is possible to ensure that the final determination of the pH of a water is always made with an indicator solution of which the pH is very close to that of the water that is being tested. Let us suppose that the first test is made with the bromothymol blue solution that is made up to pH 6.2, and that the colour so obtained corresponds to pH 6.45. The next test is made with indicator made up to pH 6.5, and this gives, let us say, a reading of 6.6. Next the bottle of pH 6.8 is used, and the reading is 6.7. It is clear from the last two results that the true pH of the water must certainly lie between 6.6 and 6.7. By a series of successive approximations of this kind, which is carried on until, of two indicator solutions whose own pH values differ by 0.4 or less, one shows a reading lower than its own pH value and the other higher, it is possible to obtain reliable determinations, with an error of not more than 0.1, of the pH of even the most feebly buffered natural waters.

The determinations were made with a Lovibond Tintometer, which is a simple comparator fitted with standard coloured glasses which correspond to nine tints within the working range of each indicator. The indicators used were bromocresol green, chlorophenol red, bromothymol blue, phenol red and thymol blue.

By means of this technique about 250 readings have been taken of the pH of Irish fresh waters. The following are the main conclusions that can be drawn from the results.

(1) The range of pH of natural waters in Ireland is from 4.1 to 8.75.

(2) Waters derived from a catchment area that is wholly or largely limestone have a pH that is always above 7.5 and usually above 8, and are well buffered. The pH of water that has been in effective contact with limestone for any length of time is always close to 8.5. The alkali reserve of a limestone water is sufficient to neutralize a large amount of humus acids. This is well illustrated by such rivers as the Inny (Co. Westmeath), which draws most of its waters from the large raised bogs of northern Westmeath, but includes also in its catchment area some grassland lying on limestone drift. At Float Bridge (just above L. Derravaragh) its water, though very brown, is alkaline, with a pH varying from 7.55 to 8.15. In L. Derravaragh itself, the shores of which are almost entirely limestone, the pH of the same water becomes raised to 8.4 and the colour is discharged, presumably by precipitation of colloidal humic compounds.

Some other determinations for limestone waters are:—

| | pH | | pH |
|-----------------------|-------------|-------------------------|-----------|
| L. Gill | 8.2 ; 8.65 | Brittas R., Co. Wicklow | ... 8.35 |
| L. Cuilin, Co. Mayo | 8.4 ; 8.55 | L. Corrib | 8.3 ; 8.4 |
| L. Gara, Co. Sligo | 8.65 ; 8.25 | L. Mask | ... 8.3 |
| L. Key, Co. Roscommon | 8.7 | L. Derg, at Killaloe | ... 8.4 |
| L. Arrow, Co. Sligo | 8.45 | L. Leane (E. shore) | 8.45 |
| R. Boyne, at Trim | 8.1 | | |

In this table, as in others, when two figures are quoted for the same water it is to be understood that different readings were obtained on two different occasions

The highest pH value that I have met with in any substantial body of water is 8.75, which is recorded for L. Labe, a small lake in S.E. Co. Sligo, derived from underground drainage of limestone mountains. Its effluent stream vanishes after about a mile in a swamp in which constant precipitation of calcium carbonate is taking place

Waters draining off basalt are also distinctly alkaline, though usually rather less strongly buffered than those from limestone. For example the stream in Glengariff, Co. Antrim, despite the fact that most of its catchment area is peat-covered, has, even at about 900 ft., a pH of 8.25. Similar figures have been recorded for other streams on the Antrim basalt.

(3) Distinctly acid waters are not nearly as widespread as is commonly supposed, and pH values lower than 5 are to be found only in water that has drained off a substantial mass of peat. I cannot agree with Southern (1932) when he says "The most acid kind of water is found in lakes which have very limited catchment basins consisting of steep slopes of non-limestone rock." Only two such lakes (Nahanagan and Acorrymore) have I found to be acid at all (and those not very strongly), many others that I have tested are virtually neutral. Pure peat-water, *i.e.* the contents of bog-holes, bog-drains, and slow streams running through large bogs, has a pH that is always close to 4.4, but as the following table shows, the figures for Connemara are higher than those for Co. Wicklow, and those for Kerry are higher still.

| | |
|--|-----------|
| Source of R. Liffey, Co. Wicklow | 4.1, 4.25 |
| Two Rock Mountain, Co. Dublin | 4.2 |
| Bog pool near Maam Cross, Co. Galway | 4.5 |
| " " " Recess " " | 4.45 |
| " " " Craiggamore " " | 4.35 |
| Bog N.E. of Brandon Mt., Co. Kerry | 4.55 |
| Bog at 1,000 ft. on W. slope of Carantuohill | 5.1 |
| Bog-pool in Glencar, Co. Kerry | 4.95 |
| Peat 2 ft. below surface of bog near Upper Lake, Killarney | 5.0 |

These figures agree with those reported by Pearsall (1938) for the pH of peat samples; he found that the average value in the Connemara blanket bog was about 4.5, which contrasts sharply with the much greater acidity (down to pH 3) characteristic of the blanket bog of Northern England. The difference is ascribed by Pearsall to the wetter summers in Ireland, which prevent temporary drying-out, with consequent oxidation. Why the peat of Kerry should be less acid still is not yet apparent.

All the samples of acid waters so far cited have been those that are in contact solely with peat. Water flowing or resting on a rocky substratum is never quite so acid, but a number of pH values between 4.6 and 5.6 have been met with in mountain streams and tarns. For example:—

| | |
|------------------------------|-----------|
| Upper L. Bray, Co. Wicklow | 4.75 |
| Effluent from Lower L. Bray | 4.65, 4.9 |
| L. Nahanagan, Co. Wicklow | 5.05; 5.5 |
| L. Acorrymore, Achill Island | .. 5.55 |
| Glenmacnass R., Co. Wicklow | 4.7; 5.0 |

Several more examples from Co. Wicklow could be given, but it is remarkable that this relatively high figure of 5.55 is the lowest pH that I have found for any lake in Western Ireland (By "lake" is implied water resting in a rocky basin, and not a mere bog-hole)

(4) In a very large proportion of the lakes and rivers of non-calcareous areas (which includes, of course, most of the mountain districts) the water is very faintly acid, neutral, or even slightly alkaline, & its pH ranges from 6.0 to 7.3. As the existence of this large class of quasi-neutral waters does not seem to be generally realized, it is worth while quoting a fairly extensive list of measurements

| Co. Cork | | Co. Kerry | |
|----------------------------|-----------|-----------------------------|-----------------|
| Owvane R., Bantry Bay | 6.9 | Owenshagh R., Lauragh | 6.2 |
| Gouganebarra L. | 7.1 | Glanmore R., Lauragh | 6.85 |
| Largest stream entering | | L. Acoose | 7.15 |
| Gouganebarra L. | 6.8 | L. Coumloughra | 7.2 |
| Bandon R., near Dunmanaway | 7.15 | Caragh R., above Lough | 6.25 |
| | | Caragh L. | 6.75 |
| | | Stream from main corrie of | |
| | | Brandon Mt. | 6.85 |
| | | Curraheen R., Slieve Mish | |
| | | Mts., at 700 ft. | 6.9 |
| Co. Tipperary | | Co. Mayo | |
| Bay L. (Knockmealdowns) | 6.4, 7.0 | Doolough | 6.9, 7.05, 7.15 |
| | | Carrowlinsky R. | 6.75 |
| | | Owenmore R., Bangor | 6.7 |
| | | Stream on Benwee Head | 7.1 |
| Co. Galway | | Co. Waterford | |
| L. Mask, S.W. limb | 6.9 | Coomshingaun L. | 7.05 |
| L. Fee | 6.9 | Stream from plateau above | |
| L. Nafooev | 6.95 | Coomshingaun | 6.8 |
| L. Inagh | 7.1, 7.2 | | |
| L. Derryclare | 7.0, 7.05 | | |
| L. Nabrucka | 7.1 | | |
| L. Oorid | 7.15 | | |
| Glendollagh | 7.05 | | |
| L. Nacoogarow | 7.1 | | |
| Ballynahinch L. | 7.0 | | |
| „ R | 6.95 | | |
| Craiggamore L. | 6.7 | | |
| Owentooey R., Recess | | | |
| (in spate) | 6.3 | | |
| Stream on N. side of | | | |
| Leckavrea Mt. | 6.95 | | |
| | | Co. Wicklow | |
| | | Stream on E. side of Djouce | 6.5 |
| | | Glendasan R., Laragh | 6.45 |
| | | Glendalough, upper lake | 6.25 |

A few of the waters in this list, such as those of Gouganebarra, Coumloughra, Doolough and Coomshingaun, are from lakes of which the catchment area is mainly steep mountainsides on which little or no peat is developed, and their neutrality is therefore not very surprising. But the great majority run off an area that is largely or at any rate partly peat-covered, and it must be concluded that the original acidity of the water is lost. The constancy with which the Connemara lakes, in particular, maintain a pH within 0.2 units of neutrality is very striking, and affords a partial explanation of the anomalies in their flora that have puzzled ecologists, such as the frequent occurrence of *Cladium mariscus*, a characteristic fen species.

These same neutral lakes, resting in granite, quartzite, or mica-schist basins, are surrounded by blanket bog, from which acid water of pH about 4.4 drains constantly into them. It would seem, therefore, that the acid must be neutralized, or precipitated, or chemically destroyed when the water flows into the lake basin and loses contact with the peat.

If it is neutralized there are two possible sources for the necessary base—veins and dykes of metamorphic limestone, intrusive basic rocks, etc., in the lake basin, or blown sand rich in calcareous debris. The latter explanation has been rather freely invoked by botanists, but I do not believe that it applies to any but a very few small lakes that are within a mile or so of extensive sandy shores. It can, in any case, be shown that the water of these Connemara lakes has a total base content that is very little higher than that of the acid bog-water which drains into them, and therefore that the loss of acidity is not principally due to neutralization by bases from whatever source. This may most easily be done by determining the weight of sulphated ash that remains after evaporation and ignition in presence of sulphuric acid. The following figures were obtained—

| | Sulphated ash, mg per litre | pH |
|----------------------------------|--------------------------------|------|
| L. Nacoogarrow | 65.4 | 7.1 |
| Adjacent bog-pools (mean of two) | 62.1 | 4.45 |

The difference, which is scarcely significant, implies the addition of only about 0.05 milli-equivalents of base per litre, which could not, in itself, account for the rise in pH .

It would seem therefore that the organic acids of the peat-water must be either precipitated or destroyed (by oxidation or otherwise) in the lake-basin. The fact that these lakes have, for the most part, clean rocky bottoms, without any appreciable deposit of organic mud, renders the first alternative unlikely, but the question clearly requires further investigation.

Attention may be drawn to the fact that in the Co. Wicklow waters examined the loss of acidity does not proceed quite so far. This is possibly connected with the greater acidity of the peat in Eastern Ireland, which has been mentioned above.

An important feature of quasi-neutral waters of this type is their extremely low buffer capacity, which is due to the virtual absence of weak acids other than carbon dioxide. Accurate determination of their pH requires, accordingly, some care. A remarkable illustration of this low buffer capacity was met with in Gougancbarra L. (Co. Cork), a mountain tarn of which the catchment area consists entirely of Old Red Sandstone. My first test gave me the astonishing figure of pH 9.0. Another sample from 200 yards along the shore showed pH 8.7. Eventually I found that this alkaline water was confined to one corner of the lake, and that the pH all along the further shore was constant at 7.1. The alkalinity must have been due to contamination from the small hotel which stands on the lake shore near my first point of sampling, and which was, presumably, discharging washing soda into the lake. This had been sufficient to raise by nearly 2 units the pH of some acres of water.

Waters of this type, which include the greater part of the mountain lakes and rivers of Ireland, are essentially dilute solutions of neutral salts, containing a quantity

of carbon dioxide determined by the partial pressure in the atmosphere, and a very minute amount of excess base, which must be derived either from solution from the soil, or from blown dust, or from adsorption of acid radicles. Sawyer (1944) has shown that many of the fresh-water lochs of Sutherlandshire are of this type, and owe their slight acidity (pH circa 6.1) entirely to carbon dioxide.

(5) There are, of course, neutral or nearly neutral waters of another type, those derived from the mixture of acid and alkaline streams, or by acid water running for a short distance over a calcareous substratum. They are, in contrast to the waters of the previous type, relatively well buffered. The R. Inny, above L. Derravaragh (mentioned earlier) belongs here, as does the middle course of the R. Liffey, in and above the new reservoir, and the middle course of most Irish rivers. A few other examples are:—

| | |
|---|------|
| L. Graney, Co. Clare | 7.4 |
| L. Levally, Co. Mayo | 7.8 |
| L. Beltra, Co. Mayo | 7.45 |
| R. Rinn, Co. Longford | 7.35 |
| Stream in Glendoo, Co. Dublin | 6.6 |
| R. Blackwater, Co. Cork (above Banteer) | 7.3 |

The occasional occurrence (as in Glendoo) of waters of this type in streams running off mountains of siliceous rock is due, of course, to the presence of calcareous glacial drift in some part of their catchment area.

(6) If water flowing from a limestone area lies in a non-limestone basin, or if a river flows successively through the two types of rock, the effect of the limestone is, as a rule predominant. The case of the R. Inny has been mentioned earlier. The reverse phenomenon is seen in L. Cullin, Co. Mayo, which lies entirely on granite. Its water, however, being derived mainly from the limestone country of E. Mayo, has a pH of about 8.5, and the figure is not perceptibly lowered by its sojourn in the granite basin.* Neutral waters, on the other hand, need to flow over limestone for only a very short distance to become distinctly alkaline. The stream which flows from the Curlew Mts. into the S.W. corner of L. Arrow (Co. Sligo) rises on Old Red Sandstone. At the point where it reaches the limestone its pH is 7.2. 300 yards lower down it has risen to 7.75; after a further 300 yards to 8.2; and finally, on entering the lake after about $1\frac{1}{2}$ miles on limestone, to 8.4. In other cases the joint result of inflow of tributaries and solution of the river-bed is to bring about much more complex gradients, as in the lower reaches of the R. Flesk (Co. Kerry) which runs through sandstone drift over limestone, and varies in its pH in the most irregular manner from 7.3 to 8.6 in the last four miles of its course.

(7) Variation of pH with season and weather is less common and less extensive than one might suppose. Flooding always lowers the pH , by increasing the amount of humus in suspension and also perhaps, in alkaline waters, by simple dilution. Frost (1939) has published figures for the Liffey, and finds that the water at Ballysmuttan varies in pH from 4.6 to 6.8, with an average of 5.6. My own determinations at the same station confirm this range of variation, but I believe it to be exceptionally

* It is, however, interesting to note that *Lobelia dortmanna*, a plant distinctly calcifuge in its distribution, grows freely in L. Cullin and the S. part of L. Conn, but not along the limestone shores of northern L. Conn. The possibility of a pH gradient in the subaqueous soils of such lakes must be born in mind.

wide, and likely to occur only in places where very acid water, like that of the upper Liffey, is changing over a short distance to alkaline, probably by the combined effects of oxidation and neutralization by calcium carbonate. Most of the other waters which I have sampled at different seasons show a range of 0.4 units or less, and some of the western lakes (e.g. Doolough) show a constancy that is especially remarkable if their low buffer capacity is taken into consideration. Even rivers can show the same constancy—the Caragh River, for example, just above the lake, sampled in July at normal summer level showed a pH of 6.2, two days later, in raging flood after a day's continuous rain, the value had risen only to 6.3.

TITRATABLE ACIDITY AND ALKALINITY.

Although pH is, in general, the most useful measure of acidity for the hydrobiologist, the titratable values of acidity and alkalinity are of some interest to the hydrographer and chemist. They enable one to distinguish between different buffer capacities in waters of the same pH, and to make, in some instances, deductions as to the nature of the acids and bases present.

In making these titrations I have somewhat modified the standard methods of water analysis. For determination of titratable *acidity* the water is titrated against *N*/200 sodium hydroxide with phenol red as indicator and an end-point at pH 7.5. In this way the carbon dioxide originally present in the sample is taken into account (*i.e.* it is neutralized as far as NaHCO_3), but re-equilibration with the atmosphere does not take place to any appreciable extent. To ensure this the titration is carried out reasonably quickly; but since the reaction $\text{CO}_2 + \text{H}_2\text{O} \longrightarrow \text{H}_2\text{CO}_3$ is a slow one, 3–5 minutes should be allowed at the end of the titration before the end-point is finally judged. For determinations of titratable *alkalinity*, it is best to determine the excess base, irrespective of the extent to which it is combined with carbonic acid. This is done by titrating against *N*. 200 HCl to an end-point at pH 5.5, using chlorophenol red as indicator. Since, during the titration, the liquid becomes supersaturated with carbon dioxide released by the added acid, it is brought to the boil shortly before the end-point, cooled, and the titration completed in a solution which is now virtually free from carbon dioxide.

The following table shows some of the values that have been obtained, the concentrations being expressed in milli-equivalents per litre.

| Source of water | pH | Acidity (mEq./l) | Alkalinity (mEq./l) |
|--------------------------------------|------|---------------------|------------------------|
| L. Bray, Co. Wicklow | 4.6 | 0.13 | — |
| Bog pool on Two Rock Mt., Co. Dublin | 4.2 | 0.32 | — |
| Source of R. Liffey, Co. Wicklow | 4.1 | 0.47 | — |
| L. Nahanagan, Co. Wicklow | 5.5 | 0.035 | — |
| Stream on Tonelagee, Co. Wicklow | 4.7 | 0.09 | — |
| L. Acorrymore, Achill Island | 5.55 | 0.115 | — |
| Doolough, Co. Mayo | 7.05 | 0.07 | 0.26 |
| L. Fee, Co. Galway | 6.9 | 0.06 | 0.085 |

(continued overleaf.)

| Source of water | pH | Acidity (mEq. l.) | Alkalinity (mEq. l.) |
|---------------------------------|------|----------------------|-------------------------|
| Stream on Leckavrea, Co. Galway | 6.9 | — | 0.34 |
| Stream on Benwee Head, Co. Mayo | 7.05 | 0.06 | 0.64 |
| Stream in Glenariff, Co. Antrim | 8.25 | — | 2.14 |
| L. Derravaragh, Co. Westmeath | 8.4 | — | 3.64 |
| R. Inny, above L. Derravaragh | 7.55 | — | 3.20 |
| L. Culin, Co. Mayo | 8.55 | — | 1.88 |
| L. Gill | 8.2 | — | 1.16 |

It will be seen that the figures for alkalinity are, in general, much higher than those for acidity, the concentration of acid even in the exceptionally acid and very dark brown sample from the source of the Liffey being less than $N \cdot 2000$. For as weak a solution as this to show a pH as low as 4.1 implies that part of the acid in solution must be relatively strong, that is to say of at least the strength of acetic acid, and the same is true, to a greater or less extent, of all the acid waters investigated. In the hopes of throwing further light on this point titration curves were prepared for the first two waters listed above: these curves are reproduced in the figure. The absence of any well-marked points of inflection in the curves suggests that at no stage in the titration are we concerned with a single acid, but that in addition to carbon dioxide both weak and relatively strong organic acids are present, the weaker being associated with brown colour, and perhaps colloidal. The divergence between the curves for boiled and for unboiled samples gives, of course, a measure of the part played by carbon dioxide in solution.

In limestone waters the lack of exact correlation between pH and titratable alkalinity is due, presumably, to the fact that in some of them the pH is lowered by organic acids that have been neutralized, but not precipitated or destroyed. The water of L. Derravaragh, with a very high calcium content (which is virtually a measure of the excess base) still retains in solution some of the acids brought down by the Inny from the peat-bogs, and has therefore a lower pH than the water of L. Culin, in which dilution of the limestone-water by nearly neutral streams flowing off granite and quartzite considerably lowers the value of the excess base but has little effect on the pH.

SODIUM AND CHLORIDE CONTENT.

The importance to most aquatic animals of the concentration of sodium chloride in the medium in which they live is not yet generally appreciated. All animals, with the possible exception of insects, require a relatively high concentration of sodium chloride in their body fluids, and it has been shown by Krogh and his pupils (for summary see Krogh, 1939) that most fresh-water animals obtain this by absorbing the salt from their environment against a concentration gradient. In many cases this power of absorption is astonishingly highly developed. the crayfish, for example, can absorb the sodium chloride so completely from a 1.1 mM. solution (0.0064%) as to reduce it to a concentration so low that the chloride cannot be detected (<0.02 mM). Similarly the stickleback can absorb sodium chloride from all solutions stronger than 0.25 mM. It would appear that for each species there is a minimum concentration below which absorption cannot be carried on—or rather cannot be carried on faster than the inevitable outward diffusion that is proceeding all the time in the opposite direction.

It would seem probable, therefore, that the sodium chloride content of the water might in some cases be a limiting factor in the distribution of fresh-water animals. A number of analyses of Irish waters have been made to elucidate this point. In particular, special attention was paid to waters derived from catchment basins composed entirely of highly insoluble rocks such as quartzite, for in these it might be expected that the amount of salts derived from weathering would be infinitesimal.

Since the estimation of chloride presents much fewer analytical difficulties than the estimation of sodium, most of the analyses were made for chloride. The water was first concentrated by evaporation to about a fifteenth of its original volume, and the chloride in the concentrate estimated by the method of Conway (1935) with minor modifications. This consists in oxidizing the chloride quantitatively to chlorine by potassium permanganate and sulphuric acid in the outer chamber of a micro-diffusion unit, and allowing it to distil over into potassium iodide solution in the inner chamber. The iodine released is estimated by titration with *N* 500 sodium thiosulphate.

The figures thus obtained are shown in the following table. (The concentration of chloride is expressed in millimoles per litre: one part of Cl in 100,000 = 0.285 mM.)

| Source | Date | W. Long- itude | Rocks of catchment basin | Cl (mM.) |
|--|---------|-------------------|------------------------------------|-------------|
| L. Bray, Co. Wicklow | 7/ '40 | 6°20' | Granite | 0.165 |
| " " " " | 11/ '46 | " | " | 0.12 |
| Source of R. Liffey | 7/ '40 | " | Peat on granite | 0.15 |
| Stream in Glendoo, Co. Dublin | 4/ '45 | " | Granite, with some drift | 0.29 |
| Glendalough, Co. Wicklow | 5/ '45 | " | Mica-schist | 0.19 |
| L. Nahanagan, Co. Wicklow | 7/ '40 | " | Granite | 0.135 |
| Stream on Tonelagee, Co. Wicklow | 5/ '45 | " | " | 0.175 |
| Stream in Glenariff, Co. Antrim | 3/ '41 | 6°5' | Basalt | 0.29 |
| L. Derravaragh, Co. Westmeath | 7/ '40 | 7°20' | Limestone | 0.34 |
| R. Inny, above L. Derravaragh | 7/ '40 | 7°25' | Limestone, largely peat-covered | 0.31 |
| Counshingaun L., Co. Waterford | 3/ '46 | 7°30' | Sandstone | 0.37 |
| L. Muskry, Galtee Mts | 6/ '45 | 8°5' | Sandstone | 0.26 |
| R. Blackwater, near Banteer, Co. Cork | 3/ '46 | 8°55' | Mainly coal measures | 0.39 |
| L. Culin, Co. Mayo | 1/ '41 | 9°10' | Limestone and granite | 0.54 |
| Stream on Leckavrea, Co. Galway | 1/ '41 | 9°35' | Quartzite | 0.48 |
| Doolough, Co. Mayo | 8/ '41 | 9°45' | Slates | 0.39 |
| L. Nacoogarow, Co. Galway | 6/ '45 | " | Mica-schist | 0.51 |
| Bog near Recess, Co. Galway | 6/ '45 | " | Peat on mica- schist | 0.60 |
| L. Craiggamore, Co. Galway | 6/ '45 | 10° | Mica-schist | 0.86 |
| L. Acorrymore, Achill I. | 7/ '40 | 10°10' | Quartzite | 0.60 |
| Stream from coomb on Brandon Mt., Co. Kerry | 3/ '46 | 10°10' | Sandstone | 0.40 |

Attention may first be drawn to the relative uniformity of the figures. The greatest concentration of chloride recorded is only seven times the least, a range which in view of the great variety of the sources of water is less than one might expect. Secondly, it will be seen that whereas there is no correlation with the type of rock from which the water flows, there is a general correlation with geographical position, the highest concentrations of chloride being found in waters near the west coast, the lowest in those furthest from it. The samples in this table can be classified as 8 eastern, 4 central, and 9 western, and the average chloride content for these three classes respectively is 0.19, 0.32 and 0.53 mM.

The explanation is simple enough—practically all the chloride in Irish fresh waters is derived from sea spray, and comes down in the rain. Owing to the greater frequency and greater force of westerly, as compared with easterly winds, the rain in western districts is more heavily charged with sea salt.

That there is a considerable transport of salt from sea to land in this fashion has been recognized for some time, but that in countries such as the British Isles, in which no place is very far from the sea, the chloride of fresh waters is derived almost entirely from sea spray and scarcely at all from the soil, has not been very generally realized. This view was long ago urged by Ackroyd (1901); and the fact that some unwarrantable conclusions drawn by Ackroyd in the same paper were successfully refuted by Joly (1901) has perhaps obscured the truth of this part of their foundation. Few figures are available for the chloride content of Irish rain. Ackroyd quotes 47.2 parts Cl per million (1.33 mM) as the annual average for Valentia, and a recalculation of the data furnished by Leonard *et al.* (1943) shows that the average for Glasnevin (where it is perhaps slightly increased by urban pollution) is 5 parts Cl per million (0.14 mM.). These figures may be compared with 10 mM. for Liverpool and 0.115 for Kew (Leonard and McVerry, 1939). It will be seen that when allowance has been made for evaporation the rain chloride alone will provide concentrations at least as high as those indicated in my analyses.

The fact that the correlation in my table between chloride concentration and western situation is not quite exact is due partly to the topographical accidents of the different stations, and partly to variation with weather and season. All published figures for rain show a very wide range of variation in chloride content from one sampling to the next, being highest for light rain in stormy weather and lowest for heavy rain in calm weather, and although this variation is largely averaged out by the mixing that takes place in the soil and in streams, it will not be wholly suppressed. A range of variation such that the maximum chloride content is twice the minimum is probably not unusual for Irish fresh waters.

So far we have considered chloride alone. What about the sodium by which it must be accompanied? Accurate estimation of sodium at a concentration of about 5 parts per million is no easy matter, but I have performed a few analyses by a modification of the method of Barber and Kolthoff (1928). 100 ml. of the water is evaporated to dryness, and the residue, after cautious ignition, taken up in 1 ml. of distilled water. The sodium is precipitated as sodium zinc uranyl acetate, and weighed in the filter-crucible in which precipitation is carried out.

| | Na present (mM) | Na " equivalent " of Cl present (mM.) | Excess Na (mM.) |
|--------------------------------|--------------------|---|-----------------------|
| L. Bray, Co. Wicklow | 0.235 | 0.14 | 0.095 |
| Source of R Liffey | 0.205 | 0.13 | 0.075 |
| L. Nahanagan, Co. Wicklow | 0.130 | 0.115 | 0.015 |
| L. Acorrymore, Achill I. | 0.57 | 0.515 | 0.055 |
| Bog-pool at Recess, Co. Galway | 0.505 | 0.515 | —0.01 |
| L. Craiggamore, Co. Galway | 0.68 | 0.74 | —0.06 |

The figures in the second column of this table show the sodium equivalent of the chloride present in the water—equivalent not in the chemical sense, but representing the amount of sodium present in the volume of sea water which would be required to provide this amount of chloride. In other words, if it be assumed that all the chloride is derived from sea water, then the concentration of sodium from this source alone is represented by the figures in the second column. It will be seen that there is a fairly close correspondence between these values and the amount of sodium actually present, which indicates that sea spray brought down in the rain is the principal source in Irish fresh waters of this element also. There is, however, in three of the samples a difference between the two figures which is probably significant, and the positive value of these figures for excess sodium indicates that the rain-borne sodium is supplemented by a smaller quantity (about 2 parts per million) which is presumably derived from weathering. In the third and fifth samples the Na/Cl ratio is almost exactly that of sea water, and the figure for excess sodium is practically zero. In the last sample, however, this figure is negative and large enough to make it unlikely, though not impossible, that it arises entirely from analytical errors. The excess of chloride over sodium in this water would imply either that a significant part of the chloride is derived from some source other than sea water, or that sodium is removed from solution by precipitation or adsorption. The latter alternative would appear the more probable.

These sodium analyses are too scanty to allow of any very far-reaching conclusions to be drawn, but it would appear that in most Irish fresh waters the sodium content is likely to be between 0.1 mM. and 0.8 mM., that it is largely derived from rain-borne sea salt, but that the rain-borne sodium is in some cases supplemented by a significant fraction derived from the rocks. The size of this fraction varies presumably with the sodium content of the rock and its rate of weathering.

The lower limit of both sodium and chloride concentrations is, therefore, determined by climatic and geographical conditions alone at a figure of about 0.1 mM., rather less than one five-thousandth of the concentration in which they occur in sea water. This dilution, great as it is, is not too great for the absorptive powers of the majority of fresh-water animals. Krogh (1939, p. 202) described a lake in central Sweden in which the concentration of chloride averages less than 0.07 mM., but which contains quite an extensive and varied fauna in which all the principal fresh-water groups are represented. It may be that some species find a concentration of 0.1 mM. uncomfortably low, at any rate for sodium (for the absorption of sodium

and chloride ions does not necessarily proceed *pari passu*), and are for this reason excluded from waters in eastern Ireland that flow off sodium-poor rocks. But such waters are likely to be of a generally oligotrophic type—poor in potassium, phosphates, and nitrates, as well as sodium, and it would be very difficult to separate the influence of these factors. In general, the supply of sodium chloride in any Irish fresh water is probably sufficient for all those animals for which it is in other respects suited as a medium.

CALCIUM CONTENT.

A few calcium analyses have been made. The higher values recorded are of little interest: hard waters may have anything from 1 mM. to about 15 mM. Ca per litre, and the variation within this range indicates chiefly how far water that has become saturated with calcium carbonate has been diluted. Waters with less than 1 mM. Ca per litre are usually reckoned as soft, and here it is of some interest to inquire into the lower limit of the calcium content, and into the source from which this calcium is derived. The figures for some very soft waters are given below.

| | Ca (mM.) |
|------------------------------|----------|
| L. Acorrymore (Achill I.) | 0.10 |
| Doolough, Co. Mayo | 0.038 |
| L. Muskry (Galtee Mts.) | 0.19 |
| Bog-pool, Recess, Co. Galway | 0.085 |
| L. Craiggamore, Co. Galway | 0.155 |
| L. Nahanagan, Co. Wicklow | 0.038 |

The lower limit of calcium concentration appears to be at about 0.03 mM. (rather more than 1 part per million). Small though this figure is, it represents from five to ten times the amount of rain-borne calcium, calculated from the chloride content of the water and the Ca/Cl ratio of sea water. Nearly all the calcium, therefore, even in these very soft waters, must be derived from weathering of rock or from blown dust or sand. Blown dust is an important factor in the mineral balance of base-poor soils in some parts of the world (*e.g.* the Alps), but it is difficult to imagine that in the wet climate of Ireland it plays a very important part. On the other hand, in a lake such as L. Acorrymore, which lies entirely in a quartzite basin, it is difficult to ascribe any measurable quantity of calcium to weathering of the rock.

For animals with calcareous shells or exoskeletons the calcium content of the water is, of course, of great importance, and concentrations of less than about 0.15 mM. are probably inadequate for proper deposition of calcium carbonate in many such species. But the question of acidity is also relevant here, and although all acid waters are soft we have seen above that some very soft waters are almost neutral. Further faunistic and chemical data are required before we can say whether acidity or lack of calcium is the factor which excludes certain molluscs and crustacea from most mountain waters.

SUMMARY.

A common source of error in the colorimetric determination of pH in badly buffered waters is discussed, and a method of eliminating it is described.

The pH of Irish fresh waters varies from 4.1 to 8.75. Values above 7.5 are found only in waters running off limestone or basalt; these have mostly a pH between 8.25 and 8.5. Only those waters that drain off substantial peat-bogs are strongly acid. The pH of such waters is 4.1–4.2 in the Wicklow Mts, 4.3–4.5 in Connemara, and 4.5–5.1 in Kerry. A very large number of mountain lakes and streams that are usually supposed to be strongly acid are, in fact, nearly neutral (pH 6.2–7.3). Such waters contain very little acid or base, and are consequently very badly buffered. Their slight acidity is due to carbon dioxide. Such neutral lakes may be surrounded by acid bogs; in such cases the acid that drains into the lake must be precipitated or destroyed; it is not neutralized.

In most waters the variation of pH with weather and season is comparatively slight.

The titratable acidity of Irish waters ranges from 0 to 0.47 mEq. per l., for a sample with pH 4.1. This indicates that fairly strong acids are present. Titration curves show no sharp inflexion. The titratable alkalinity (excess base) may be as high as 3.6 mEq. per l., and probably higher.

The concentration of chloride is from 0.12 to 0.86 mM. (4.3 to 30.6 parts per million). It is probably all derived from rain-borne sea spray. The highest concentrations are found near the west coast, the lowest in the east. The figures for sodium (0.13 to 0.68 mM.) show a fairly close correlation with those for chloride, suggesting that most of the sodium is also rain-borne, but in some waters a significant fraction seems to be of terrestrial origin.

The calcium concentration in the softest waters may be as low as 0.038 mM. (1.5 parts per million).

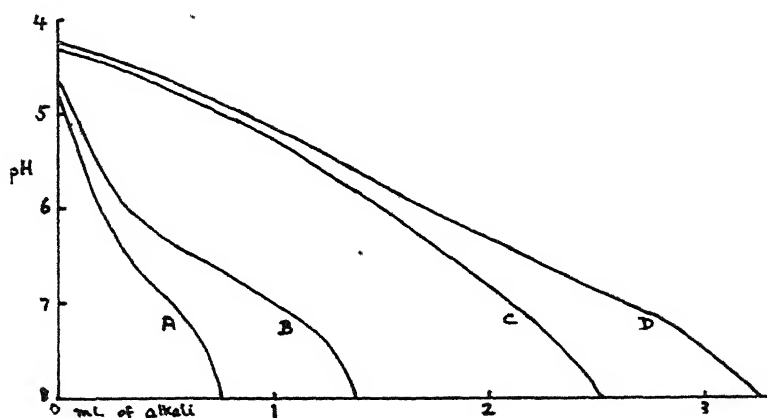


FIG. 1. Titration curves, against N/200 alkali, of 50 ml. samples of:—A, Water from L. Bray, boiled to expel CO_2 . B, the same, unboiled, C, water from a bog-pool on the Two-Rock Mountain, boiled to expel CO_2 . D, the same unboiled.

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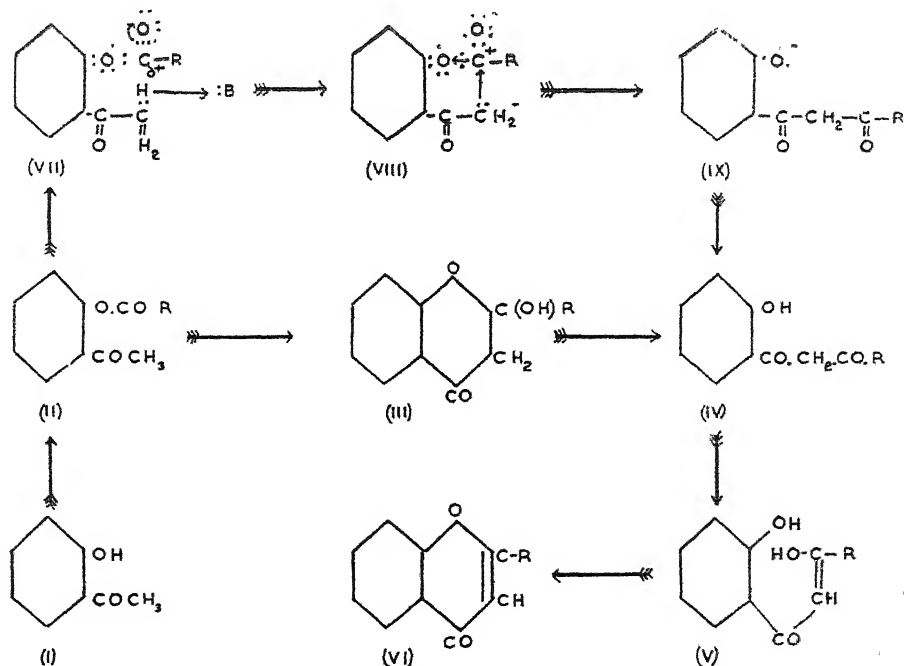
TRANSFORMATION REACTIONS. I. THE MECHANISM OF THE
TRANSFORMATION OF *o*-AROYLOXYACETOARONES INTO
o-HYDROXYDIAROYLMETHANES, AND THE
SYNTHESIS OF FLAVONES.

By B. G. DOYLE, FIONNGHUALA GÓGAN, J. E. GOWAN, JOHN KEANE AND T. S. WHEELER.

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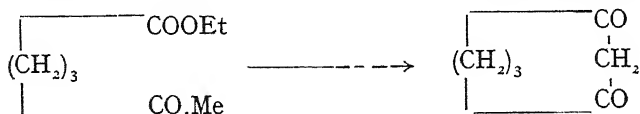
INTRODUCTION

In 1933 Baker (1, 2) found that anhydrous potassium carbonate in hot benzene or toluene, effected the transformation of *o*-aroyloxyacetoarones (II), into the corresponding *o*-hydroxydiaroylmethanes (IV); with, he suggested, the intermediate formation of the hydroxyflavanone (III). The same rearrangement, which is of synthetical importance, owing to the ready and sometimes spontaneous convertibility of (V), the enolic form of (IV), into a flavone (VI), was independently discovered by Venkataraman and his collaborators (5, 14, 15), the reagent used by them being sodamide in dry ether



Mechanism of the Transformation

Later Virker and Wheeler (24) and Ullal, Shah and Wheeler (21) found that sodium in ether or boiling toluene, and sodium ethoxide in alcohol, were also effective reagents for this transformation, and in view of these results Ullal and Wheeler (20) tentatively suggested, that the reaction might be regarded as an internal Claisen condensation, between an ester and a ketone, differing from the Dieckmann reaction in that the alcohol (in this case a phenol) eliminated remains



part of the molecule undergoing transformation. If this view is correct, the mechanism of the reaction involves a nucleophilic displacement of oxygen on carbon by the carbanion formed by the proton attraction of the basic* transforming agent thus:—

- (a) The base (:B) removes a proton from the methyl carbon, which being adjacent to the carbonyl group, contains "ionizable" hydrogen (VII).
- (b) The carbonyl carbon of the ester, which is electrophilic by resonance, tends to accept the lone pair of electrons formed on withdrawal of the proton. As negatively charged carbon approaches negatively charged oxygen recedes, with the intermediate formation of a transition state (VIII), which corresponds to Baker's intermediate compound (III).
- (c) Elimination of phenol occurs with the production of a diketone, which separates as a salt of the metal ion present (IX). Baker (1) showed that in the salt so formed, the metal ion is probably attached to the diketone portion of the molecule.
- (d) The addition of a proton produces (IV) †

Results with various Transforming Agents

Support for the mechanism now suggested, is provided by its success in predicting that, as will be seen from Table I and the experimental section, a variety of strong bases (anions of weak acids) produce rearrangement. The reaction solvent used is important; acetone and dioxan are useful, but pyridine is generally the most satisfactory. This is to be expected as the basic "atmosphere" it provides assists in the separation of a proton from the methyl carbon. Indeed it has been found that flavone (VI; R=Ph) is produced directly when *o*-benzoyl-oxyacetophenone (II; R=Ph) is heated with pyridine in a sealed tube at 275°, *o*-hydroxydibenzoylmethane (IV; R=Ph) being presumably formed intermediately, though this cannot be regarded as settled. Cyclization of (II) through (VIII), or (III) as Baker suggested, could occur forming (VI) without production of (IV).

* The word "base" is used in a general sense, *i.e.*, a species having a tendency to add on a proton (4).

† The theory of the intermolecular Claisen condensation together with a list of reagents is given by Hauser and Hudson (11). See also Watson (25, 26); Lapworth (13); Hann and Lapworth (9).

TABLE I

Yields obtained in the Transformation of *o*-Benzoyloxyacetophenone (II, R=Ph) into *o*-Hydroxydibenzoylmethane (IV, R=Ph)¹

| Results with alkali metals and their carbonates | | | | | | | Results with other transforming agents | | | | |
|---|-------------------|--|---------|--------|---|---|--|--------------------|------------------------------|----------------------------|--|
| Metal M | Electronegativity | Yields (%) of crude product ² with M in solvent shown | | | Yields (%) of crude product ³ with M ₂ CO ₃ in pyridine pK (H ₂ CO ₃) = 6.5 | Time (hrs.) of heating with M ₂ CO ₃ under reflux | Transforming agent | pK of related acid | Reaction solvent | Yield (%) of crude product | m p (crude) except where recrystallising solvent is shown ⁴ |
| | | Pyridine | Acetone | Dioxan | | | | | | | |
| Li | 1.0 | 48 | 40 | 48 | No reaction | 6 | Na salt of (I) | — | Pyridine | 80 | 117–119° (alcohol) |
| Na | 0.9 | 56 | 51 | 62 | 65 | 6 | KOH ⁶ | 16 | Pyridine | 83 | 119.5–120° |
| K | 0.8 | 69 | 70 | 52 | 74 ⁷ | 1 | NaOH | 16 | Pyridine | 74 | 119–120° |
| Rb | 0.8 | 69 | — | 52 | 82 | 0.5 | NaH* | — | Pyridine | 72 | 113–115° |
| Cs | 0.7 | — | — | — | 75 | 0.25 | NaOPh | 10 | Pyridine | 70 | 111–115° |
| | | | | | | | Na ₂ O ₂ | 12 | Pyridine | 64 | 118–119° |
| | | | | | | | " | " | Acetone | 42 | 117–120° |
| | | | | | | | " | " | Dioxan | 40 | 118–119° |
| | | | | | | | NaC(Ph) ₃ * | 33 | Ether | 64 | 119–120° (alcohol) |
| | | | | | | | NaOEt* | 18 | Pyridine | 60 | 113–116° |
| | | | | | | | " | " | Pyridine–alcohol (3:2) | 31 | 119–120° |
| | | | | | | | NaOMe* | 16 | Pyridine | 43 | 117–119° |
| | | | | | | | " | " | Acetone | 50 | 117–120° |
| | | | | | | | " | " | Dioxan | 48 | 117–119° |
| | | | | | | | " | " | Alcohol | 18 | 103–116° |
| | | | | | | | NaNH ₂ * | — | Pyridine | 56 | 117–118° |
| | | | | | | | " | — | Dioxan | 50 | 118–119° |
| | | | | | | | " | — | Pyridine–triethylamine (3:1) | 50 | 117–119° |
| | | | | | | | " | — | Pyridine–acetone (1:1) | 54 | 118–119° |
| | | | | | | | " | — | Liquid ammonia | 17 | 119° (alcohol) |
| | | | | | | | Ethyl sodioacetate | 11 | Pyridine | 50 | 114–117° |
| | | | | | | | PhMgBr ⁸ | — | Ether | 15 | 120° (alcohol) |
| | | | | | | | MeMgI ⁸ | — | Ether | 8 | 119° (alcohol) |
| | | | | | | | Sodium aluminate | — | Pyridine | 41 | 115–116° |
| | | | | | | | MeCOOK | 4.7 | Pyridine | 24 | 116–118° |
| | | | | | | | PhCOOK | 4.2 | Pyridine | 10 | 120° (alcohol) |

1. For reaction conditions see "Experimental"

2. The m p of the crude product was not below 115° Mixed m p with (IV, R=Ph) was nearer to 120°

3. The m p of the crude product was not below 118° Mixed m ps. were satisfactory

4. Mixed m ps with (IV, R=Ph) were satisfactory Addition of 2% and of 10% of (II, R=Ph) reduces the initial m p. from 120° to 117° and 111° respectively

5. Na in ether gave a yield of 50% (24)

6. KOH gave a 79% yield with stirring for 1½ hours at room temp.

7. K₂CO₃ in toluene gave a yield of 32% (1) If 10% of water is added to the pyridine solvent the yield is greatly reduced

8. Other Grignard reagents have been used previously in intermolecular Claisen condensations

*Previously used for intermolecular Claisen condensations

Alkali metals Turning to the examination of the semi-quantitative results in Table I, it will be observed that with the alkali metals the yields increase with the basicity of the metals used, lithium giving generally the lowest and rubidium (metallic caesium was not available) the highest yield; the result with sodium in dioxan is anomalous. The electronegativity values (power of attracting electrons) shown in the Table are taken from Pauling (18), the lower this value the greater the basicity of the metal. The fact that a graph of yield of *o*-hydroxydibenzoylmethane against the reciprocal of the electronegativity (Fig. 1.) is a straight line with pyridine and acetone as solvents may be of significance, as the action of the metal in effecting transformation is to be referred to the displacement of hydrogen from the acetyl methyl group to form an ion of the type shown in (VIII).

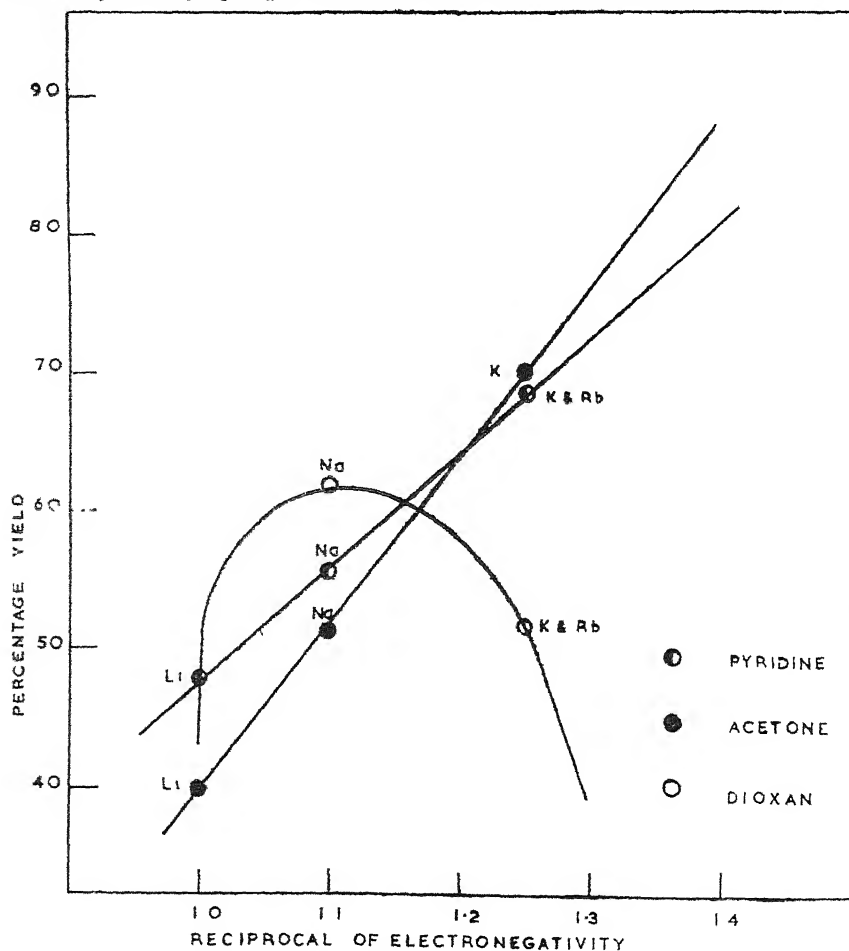


FIG. 1.

Alkali metal carbonates. Here also the yield tends to increase with the basicity of the metal ion. Lithium carbonate is inactive, while with caesium and rubidium carbonates the transformation is rapid, as indicated by the formation of the yellow salt of *o*-hydroxydibenzoylmethane. It should be noted that potassium hydrogen carbonate gives a yield of only 25%, and sodium hydrogen carbonate 10%, over a period of three hours, these salts are much less effective than the normal carbonates.

Other transforming agents. In this section of Table I the approximate pK value for the acid constant of the anion concerned, which is given by

$$K = [\text{OH}_2][\text{:B}]/[\text{H:B}]; \text{pK} = -\log K.$$

has been shown, where a figure is available. (See also Fig. 2.). The greater the value of the pK, the weaker the acid and the stronger the conjugate base; the triphenylmethyl anion (pK=33) is one of the strongest bases known. Fig. 2 shows that there is a tendency for yield to increase with pK; the results with the alkoxides have been omitted as they are reduced by alcoholysis.

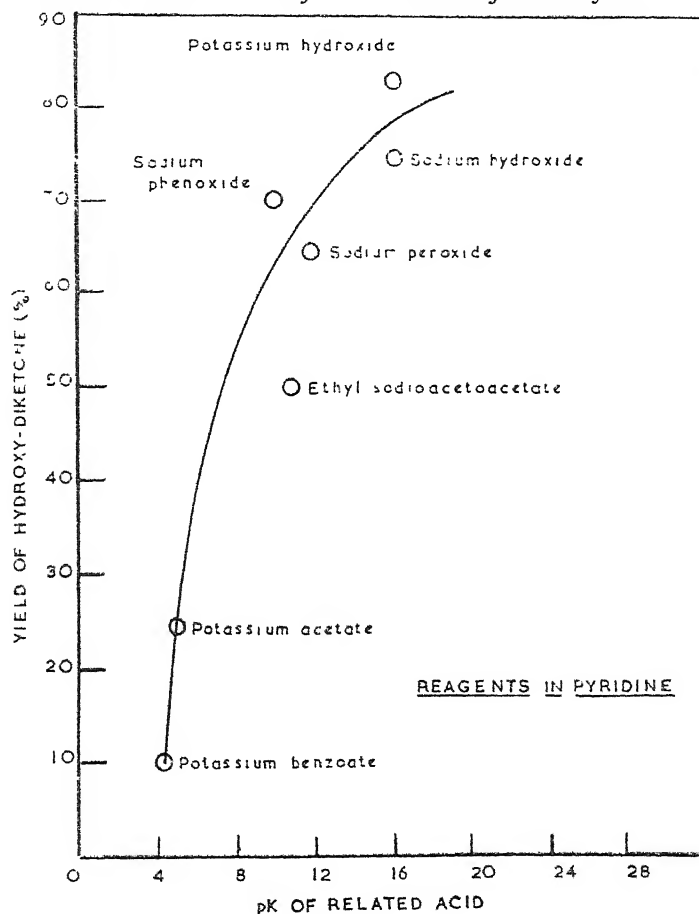


FIG. 2.

It will be observed that some of the effective transforming agents have previously been used in the Claisen intermolecular condensations (See Hauser and Hudson, (11); for the use of sodium hydride see Hansley and Carlisle, (10); Swamer and Hauser, (19)). It is hoped to examine the effect in such inter-molecular condensations, of the other transforming agents here employed.

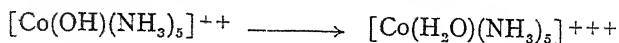
Taking the results in detail, the high yield obtained with the sodium salt of *o*-hydroxyacetophenone is probably to be attributed to the fact that with it alcoholysis merely reforms *o*-benzoyloxyacetophenone, the ester undergoing transformation. The relatively low yields obtained with alkoxides, especially in the presence of alcohol, can be referred to alcoholysis—this phenomenon is discussed

below in connection with nitro-esters. Ullal, Shah, and Wheeler, (21), however, found sodium ethoxide in alcohol a useful transforming agent with esters of hydroxyacetophenones. Sodium phenoxide, where presumably there is less tendency to alcoholysis, gave a good yield in the present instance. The smooth transformation obtained with anhydrous sodium and potassium hydroxides may be linked with the basic strength of hydroxylion. The value of sodium hydride in Claisen condensations is, to some extent, due to the fact that the hydrogen anion (H^-) combines with the proton (H^+) it removes, to form molecular hydrogen, which escapes, so that further undesirable reaction is avoided—an advantage also obtained with alkali metals.

Sodium peroxide, which here gives a yield of over 60%, does not often figure as a condensing agent. The exceptional basic strength of the triphenylmethyl anion makes it a valuable reagent, but it leaves triphenylmethane to be removed. The yields given by sodamide (about 50%, except in liquid ammonia) are greater than those obtained by Mahal and Venkataraman (15) with a number of 1-aroxy-2-acetophenones, using ether as solvent. Ethyl sodioacetate gives a moderate yield: with it alcoholysis is unlikely owing to the size and resonance of its ion. The basic strength of Grignard compounds ($\text{R}^-\text{Mg}^+\text{Hal}^-$) is due to the tendency of the hydrocarbon radical to acquire a proton, but their great reactivity produces side reactions. The effectiveness of sodium aluminate is interesting, as it seldom functions as a reagent in organic chemistry.

The results with potassium acetate and potassium benzoate indicate that this transformation, as Baker (1) suggested, provides a clue to the mechanism of salt-anhydride aroylation of *o*-hydroxyacetophenones to chromones. This aspect of the transformation will form the subject of a subsequent paper.

Using pyridine as solvent transformation was, under the experimental conditions employed, not obtained with the following reagents:—aluminium alkoxides; calcium metal; calcium carbide; calcium hydroxide; magnesium metal, sodium bismuthate; sodium borate; sodium cyanide; hydroxypentamminocobaltic chloride. The basic action of the latter salt involves the reaction:—

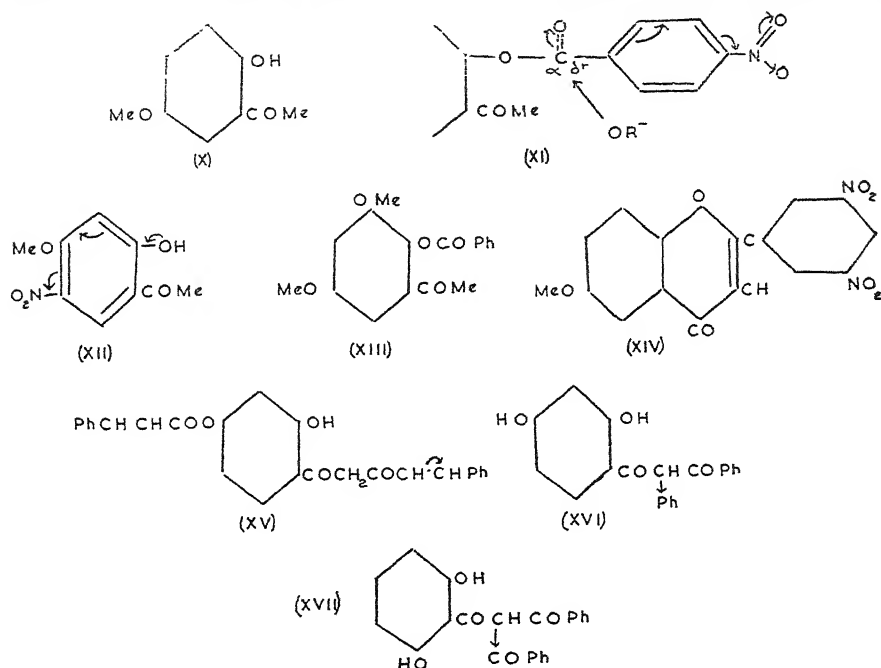


Sodamide in piperidine or quinoline was also ineffective.

Taken as a whole, the results given above indicate that the transformation under discussion involves a base-catalysed intramolecular condensation of the Claisen type.

Transformation of Nitro-esters.

Support is provided for these views, by results which have been obtained with various nitrobenzoic esters of (1) and of 2-hydroxy-5-methoxy-acetophenone (X). The presence of the nitro-group in compounds of the type (XI) renders the C_a more electrophilic, thus facilitating not only transformation but also reaction with the anion of the transforming agent. With the 3:5-dinitrobenzoic ester of (1), sodium ethoxide gave ethyl 3:5-dinitrobenzoate, and sodamide, 3:5-dinitrobenza-



mide. Transformation of nitro-esters can, however, be effected with the sodium salts of (I) and (X), and with sodium hydroxide, sodium hydride, sodium peroxide, and ethyl sodioacetoacetate, though the peroxide produced violent reaction with the dinitro-esters. It should be mentioned that Ullal, Shah, and Wheeler, (22) found that sodium ethoxide in alcohol caused alcoholysis without transformation with esters of (XII) in which the nitro-group in the acetophenone nucleus renders the C_α (See XI) electrophilic.

Effect of methoxyl groups.

The tendency of the methoxyl group in esters of (X) to release electrons to the *p*-side chain, and so reduce the electrophilic nature of the ester carbonyl carbon, hinders transformation. Thus sodium methoxide did not transform the benzoic ester of (X), although with sodium peroxide a yield of 76% of diketone was obtained. Similarly Baker, Brown, and Scott, (3) were unable to transform 2-benzoyloxy-3:5-dimethoxyacetophenone (XIII) with sodamide in toluene. In the present research it was not found possible to transform the β -phenylpropionic ester of (I)—presumably the anionoid effect of the alkyl chain in the acid radical reduces the electrophilic action of the ester carbonyl carbon. On the other hand the cinnamic and *m*-nitrocinnamic esters of (I), in which the anionoid effect is reduced, were both readily transformed to the corresponding *o*-hydroxydiketones, which were cyclized respectively to 2-styrylchromone—an authentic sample of which was prepared from 2-methylchromone (28) and benzaldehyde, by the method of Cheema, Gulati and Venkataraman (8)—and 2-(*m*-nitrostyryl)chromone.

As a matter of interest attempts were made to transform the *o*-toluenesulphonic ester of (I) but without success—further experiments with sulphonic esters are in hand.

Cyclization of 1:3-Diketones.

A variety of reagents have been used for this cyclization, of which three of the most useful, concentrated sulphuric acid, and acetic acid containing sodium acetate or a trace of mineral acid, are due to Wittig (27) and Baker (1). Hydrogen bromide in acetic acid is also satisfactory (21, 24). Pyridine with a trace of mineral acid has now been found efficient, but shows no advantage over the reagents previously employed.

Preparation of flavone. The facts that yields of about 80% can now be obtained in the transformation of *o*-benzoyloxyacetophenone into *o*-hydroxydibenzoylmethane makes it possible to prepare flavone from *o*-hydroxyacetophenone (I-V; R=Ph) with a yield of over 40%. Furthermore the sequence of operations can be carried through within two hours, so that the route thus provided for the preparation of flavone is, perhaps, more satisfactory than those hitherto available. For example, direct arylation of (1) by treatment with benzoic anhydride and sodium benzoate for eight hours gives a yield of flavone of less than 10% (7), while selenium dioxide dehydrogenation during twelve hours of *o*-hydroxyphenylstyrylketone ($C_6H_4(OH).CO.CH:CHPh$), which must first be prepared from *o*-hydroxyacetophenone, gives flavone in a 43% yield (16).

The Effect of Nitro-groups on Cyclization.

The presence of nitro-groups in the acid portion of an ester of *o*-hydroxyacetophenone facilitates cyclization of the related *o*-hydroxydibenzoylmethane. Thus, the 3:5-dinitrobenzoic ester of (X), when treated with the sodium salt of that phenol in pyridine gave on acidification 3':5'-dinitro-6-methoxyflavone (XIV), and not the expected diketone. Again the diketone from the *m*-nitrobenzoate of (1) was cyclized by heating above the melting point. These and the facile cyclization of 3:5-dinitro-2'-hydroxydibenzoylmethane (Table 4) are possibly due to the -I, -T, effects of the nitro-group, which renders a methylene hydrogen of the 1:3-diketone more labile, thus promoting enolization and consequently cyclization. A survey of the literature confirms this view, since in general when the intermediate diketone cyclizes readily it contains an electrophilic group. Compounds (XV) and (XVI), (1) and (XVII) (2) provide examples of diketones which readily yield the corresponding chromones.

Nitroflavones. The preparation of simple nitroflavones was attempted by Bogert and Marcus (6), who by the direct nitration of flavone obtained a mixture of 2'-, 3'-, and 4'-nitroflavone, which could not be separated. The corresponding aminoflavones were, however, prepared from the mixed product in a pure state by reduction. 2'- and 4'-Nitro- and 3':5'-dinitroflavone have now been synthesised by the transformation reaction. Virkar (23), had previously obtained 3'-nitroflavone. 3':5'-Diaminoflavone and its diacetyl derivative have also been prepared

EXPERIMENTAL

Preparation of o-Hydroxyacetophenones and their sodium salts.

o-Hydroxyacetophenone (1) (17) and 2-hydroxy-5-methoxyacetophenone (X) (3) were prepared according to the methods given by the authors cited. The sodium salts of these phenols separated when a molecular proportion of sodium methoxide

was shaken with a solution of the phenol in dry ether (29). The precipitated salts were dried under reduced pressure.

Preparation of acid chlorides.

The acid chlorides required for the preparation of the esters of *o*-hydroxyacetophenones (I and X) were, if not available, obtained by removing under reduced pressure excess of reagent from a solution of the acid in thionyl chloride, which had been heated under a reflux until the evolution of hydrogen chloride and sulphur dioxide ceased, moisture being excluded.

Preparation of *o*-aroyloxyacetophenones.

A solution of the phenol in pyridine (about 3 ml. of solvent per g.), which had been treated with the relevant acid chloride (1.1 mol.) and either heated at 100° for 15-30 min. or preferably kept at room temperature overnight, was poured into excess of aqueous mineral acid (up to 5%). The solid product, after it had been washed with aqueous sodium hydroxide (2%) until it gave no colour in alcoholic solution with aqueous ferric chloride, with aqueous acetic acid (10%), and with water, was recrystallised from a suitable solvent.

As *o*-(β -phenylpropionyl)oxyacetophenone did not solidify when the reaction mixture from (I) and the acid chloride were poured into dilute acid, an ethereal solution of the resulting oil was washed with aqueous sodium hydroxide (2%) and dried over anhydrous sodium sulphate. The liquid remaining after removal of the solvent solidified after distillation at 200-220°/1mm.

Of the esters prepared in the course of this work *o*-benzoyloxyacetophenone (ester serial No. 1) (1: see below under *Preparation of flavone from o-hydroxyacetophenone*) and *o*-(3-nitrobenzoyloxy)acetophenone (ester serial No. 3) (23) have already been described. The remainder, which are new, are given in Table 2.

TABLE 2
Esters of *o*-hydroxyacetophenone (I) and of 2-hydroxy-5-methoxy-acetophenone (X).

| Phenol | Acyl radical of ester | Ester serial No | m p | Crystallising solvent | Formula | Found % | | | Required % | | |
|--------|---------------------------|-----------------|---------|--|---|---------|-----|-----|------------|-----|-----|
| | | | | | | C | H | N | C | H | N |
| I | 2-Nitrobenzoyl- | 2 | 123-5° | Alcohol | C ₁₅ H ₁₁ O ₅ N | 63.3 | 4.1 | 4.7 | 63.2 | 3.9 | 4.9 |
| I | 4-Nitrobenzoyl- | 4 | 91-2° | Aqueous alcohol | " | 63.3 | 3.9 | 5.1 | " | " | " |
| I | 3,5-Dinitrobenzoyl- | 5 | 131-3° | Alcohol-acetone | C ₁₅ H ₁₀ O ₇ N ₂ | 54.0 | 3.4 | 9.0 | 54.6 | 3.0 | 8.5 |
| I | β -Phenylpropionyl- | 6 | 36 | Methyl alcohol-light petroleum (60-80°) | C ₁₇ H ₁₆ O ₃ | 76.7 | 6.0 | — | 76.1 | 6.0 | — |
| I | Cinnamoyl- | 7 | 73° | Alcohol | C ₁₇ H ₁₄ O ₃ | 76.1 | 5.4 | — | 76.7 | 5.3 | — |
| I | 3-Nitrocinnamoyl- | 8 | 122-4° | Acetone and ligroin | C ₁₇ H ₁₃ O ₅ N | 65.6 | 4.1 | 5.3 | 65.6 | 4.2 | 4.5 |
| I | 2-Toluenesulphonyl- | 9 | 90-1° | Aqueous alcohol | C ₁₈ H ₁₄ O ₄ S | 61.5 | 5.0 | — | 62.1 | 4.8 | — |
| X | Benzoyl- | 10 | 55-7° | Aqueous acetic acid and light petroleum (40-60°) | C ₁₆ H ₁₄ O ₄ | 71.1 | 5.2 | — | 71.1 | 5.2 | — |
| X | 4-Nitrobenzoyl- | 11 | 146-7° | Alcohol (charcoal) | C ₁₆ H ₁₃ O ₆ N | 60.9 | 3.8 | 4.3 | 61.0 | 4.1 | 4.4 |
| X | 3,5-Dinitrobenzoyl- | 12 ¹ | 139-40° | Alcohol | C ₁₆ H ₁₂ O ₈ N ₂ | 53.2 | 3.4 | 7.8 | 53.3 | 3.3 | 7.8 |
| X | Cinnamoyl | 13 ¹ | 90° | Alcohol | C ₁₈ H ₁₆ O ₄ | 72.5 | 5.2 | — | 73.0 | 5.4 | — |

¹ Compounds (12) and (13) formed yellow crystals, the remainder were colourless.

Action of heat on a pyridine solution of o-benzoyloxyacetophenone.

The solution (1 g. in 15 ml.) was heated at 275° in a sealed tube for 24 hours and the product was mixed with an excess of dilute hydrochloric acid. Crystals of flavone (m.p. 93-4° and mixed m.p. 95-6°, with an authentic specimen, m.p. 97°) eventually formed on the surface of the tarry precipitate.

Preparation of 1:3-Diketones.

Transformation of o-benzoyloxyacetophenone into o-hydroxydibenzoylmethane. (Table 1).

Alkali metals. A solution of *o*-benzoyloxyacetophenone (m.p. 87°; 0.5 g.) in pyridine (ca 10 ml.) was treated with the alkali metal (1 atom) in small lots, and the mixture was heated under reflux until reaction ceased; the times required were: Li, 30 min.; Na, 15 min.; K and Rb, 7 min. The cooled product was acidified with dilute acetic acid (10%; caution, unchanged metal) and the precipitated *o*-hydroxydibenzoylmethane removed by filtration.

The procedure with acetone and dioxan was similar. Using the former solvent reaction was complete in 10 min. There was some interaction between potassium and acetone, but this did not interfere with the transformation. With rubidium, however, the vigorous reaction between the metal and the solvent caused inflammation. Reaction in dioxan as solvent was complete in 20 min.

Alkali metal carbonates. A slight excess (more than 1 mol.) of the carbonate was employed, and heating under a reflux continued for the times shown in Table 1. The product was separated by the addition of dilute acid.

Other transforming agents. Generally a solution of the ester (0.5-1 g.) in pyridine (10-20 ml.) or other solvent was heated with agitation at up to 115° for about 15 min. with the transforming agent (ca. 1.2 mols.), and the liquid acidified with dilute acetic acid (10%) in the cold to precipitate the diketone. Care in acidification is necessary with alkali hydroxides, sodium hydride, sodium peroxide, and sodamide. (See also below under *Preparation of flavone from o-hydroxyacetophenone.*) Modifications in the general method required with some of the reagents used are set out below:—

Triphenylmethylsodium. A solution of *o*-benzoyloxyacetophenone (0.5 g.) in anhydrous ether (10 ml.) contained in a reaction flask filled with nitrogen was treated with a solution of triphenylmethylsodium, prepared, stored, and delivered into the reaction flask in an atmosphere of nitrogen using the procedure of Hauser and Hudson (11). Addition of the reagent was stopped when the red colour of its ethereal solution was no longer discharged. The reaction mixture was acidified with aqueous acetic acid (10%), and the ethereal layer extracted with aqueous sodium hydrogen carbonate (2%) to remove acid, and with aqueous potassium hydroxide (2%) until colourless. The extracted diketone was precipitated from the alkaline solution by the addition of dilute acid and recrystallised from alcohol.

Sodamide in liquid ammonia. Liquid ammonia was added from a cylinder to *o*-benzyloxyacetophenone (1 g.) and an excess of sodamide in a Dewar flask, and the mixture was kept for 48 hours, by which time the ammonia had evaporated. The residue was treated with water (caution) and the solution acidified with dilute acetic acid to precipitate a brown tar, which on recrystallisation from alcohol yielded *o*-hydroxydibenzoylmethane.

Grignard reagents. A solution of *o*-benzyloxyacetophenone (1 g.) in anhydrous ether (12 ml.) was added to anhydrous ether (5 ml.) containing a Grignard reagent prepared from magnesium ribbon (0.1 g) and bromobenzene (0.7 g), and the mixture was heated under a reflux for 5 min. and acidified with dilute hydrochloric acid. The diketone was recovered from the ethereal layer as with triphenylmethyl-sodium. A similar reaction was carried out with a Grignard reagent prepared from methyl iodide.

Sodium aluminate. A solution of *o*-benzyloxyacetophenone (0.5 g.) in pyridine (15 ml.) containing an excess of sodium aluminate was heated under a reflux for 10 min., and cautiously acidified with sulphuric acid (50%). The supernatant diketone was, as far as possible, separated mechanically, and the residual liquid and solid were extracted with ether to recover a further quantity of diketone.

Potassium benzoate and potassium acetate. With these salts heating under reflux was continued for eight hours. Using potassium acetate the mixture was acidified to precipitate the diketone. With potassium benzoate the precipitate obtained on acidification was dissolved in ether, and the solution extracted with aqueous sodium hydrogen carbonate to remove benzoic acid. The solvent was then volatilized.

Preparation of 1:3-Diketones other than o-Hydroxydibenzoylmethane.

Table 3 gives particulars of the transformation of a number of esters of *o*-hydroxyacetophenones using either the method given above under *Other transforming agents*, (Method A) or a modification, in which the solution of the ester in pyridine was kept in contact with the reagent at room temperature for 24-48 hours (Method B). It was not found possible to transform either *o*-(β -propionyloxy)acetophenone (ester serial No. 6) or *o*-toluenesulphonyloxyacetophenone (ester serial No. 9). 2-(3':5'-Dinitrobenzyloxy)-6-methoxyacetophenone (ester serial No. 12) yielded the corresponding *flavone* directly on transformation.

TABLE 3

Hydroxy-1,3-diketones prepared by the Transformation of the corresponding Esters.

| Ester serial No. | 1,3-Diketone | Diketone serial No. | m p and solvent | Formula and description | Elements | Found % | Required % | Method used, transforming agent ¹ and crude yields |
|------------------|---|---------------------|---|--|-------------|--------------------|--------------------|---|
| 2 | 2-Nitro-2'-hydroxy-dibenzoylmethane | 1 | 162-3 Dioxan | C ₁₅ H ₁₁ O ₅ N Yellow crystals | C H N | 63.3 4.1 4.9 | 63.2 3.9 4.9 | B NaOH, 36%, NaH, 80%, NaA, 50%; Na ₂ O ₂ , 60% |
| 3 | 3-Nitro-2'-hydroxy-dibenzoylmethane (23) | 2 | 154-7° Alcohol-acetone and aq. acetic acid | — | — | — | — | B NaAr, 60% |
| 4 | 4-Nitro-2'-hydroxy-dibenzoylmethane | 3 | 198-201° Benzene | C ₁₅ H ₁₁ O ₅ N Yellow crystals | C H | 63.4 4.0 | 63.2 3.9 | A NaA, 47% |
| 5 | 3,5-Dinitro-2'-hydroxy-dibenzoylmethane | 4 | 227-8° | C ₁₅ H ₁₀ O ₇ N ₂ Golden brown plates | C H N | 55.1 3.4 8.0 | 54.6 3.0 8.5 | B NaAr, 70%, NaH & NaA also effective ² |
| 7 | 2-Hydroxybenzoyl-cinnamoylmethane | 5 | 134° Alcohol | C ₁₇ H ₁₄ O ₃ Pale yellow crystals | C H | 77.1 5.0 | 76.7 5.3 | A NaOEt in alcohol (no pyridine), 25% |
| 8 | 2-Hydroxybenzoyl-3'-nitrocinnamoylmethane | 6 | 158°-60° Benzene and glacial acetic acid | C ₁₇ H ₁₃ O ₅ N Yellow rectangular crystals | C H N | 64.9 4.4 4.9 | 65.6 4.2 4.5 | B NaAl, 70% |
| 10 | 2-Hydroxy-5-methoxy-dibenzoylmethane | 7 | 81° Aq. alcohol | C ₁₆ H ₁₄ O ₄ Bright yellow needles | C H | 70.7 5.4 | 71.1 5.2 | B Na ₂ O ₂ , 76%, NaOMe did not effect transformation |
| 11 | 4-Nitro-2'-hydroxy-5'-methoxydibenzoylmethane | 8 | 173-4° Alcohol | C ₁₈ H ₁₃ O ₆ N Yellow crystals | C H N | 60.2 4.4 4.1 | 61.0 4.1 4.4 | B NaA, 30%; NaOMe, 12% (alcoholysis). |
| 13 | 2-Hydroxy-5-methoxy-benzoylcinnamoylmethane | 9 | 95-7° Aq. alcohol | C ₁₈ H ₁₆ O ₄ Dark yellow crystals | C H | 72.6 5.2 | 73.0 5.4 | A, NaOMe, 60% |

¹ NaA = Ethyl sodioacetoacetate, NaAr = Na salt of *o*-hydroxyacetophenone

² When the transformation of 3,5-dinitrobenzoyloxyacetophenone was attempted using sodium ethoxide in pyridine, alcoholysis occurred, and ethyl 3,5-dinitrobenzoate, m.p. 90-1° (lit. 93-4°), (Found: C, 44.8; H, 3.3; Calculated for C₉H₈O₆N₂, C, 45.0; H, 3.3%) was obtained. The use of sodium peroxide caused a violent reaction, while triphenylmethylsodium in chloroform or pyridine gave no useful result. 3,5-Dinitrobenzamide m.p. 183° (lit. 183°) was obtained, when sodamide in pyridine was employed.

Cyclization of Hydroxy-1,3-diketones.

In general a solution of the diketone in glacial acetic acid containing a few drops of mineral acid was heated to boiling, cooled, and diluted with water to precipitate the chromone. Baker (1), obtained quantitative yields of flavone in the

cyclization of *o*-hydroxydibenzoylmethane with concentrated sulphuric acid in the cold, or with glacial acetic acid containing sodium acetate at the boiling point for 30 min

Table 4 gives particulars of the chromones, mainly flavones, prepared in the course of the present work.

TABLE 4
Chromones prepared by Cyclization of Hydroxy-1,3-diketones

| Diketone serial No. | Chromone | m p and crystallizing solvent | Formula and description | Elements | Found % | Required % | Yield % | Method of cyclization ¹ |
|---------------------|-------------------------------------|--|---|--|---------------------------|---------------------------|---------|------------------------------------|
| 1 | 2'-Nitroflavone | 180-2° Dioxan | C ₁₅ H ₉ O ₄ N White prisms | C H N | 67.3 3.4 5.2 | 67.4 3.4 5.2 | 50 | a |
| 2 | 3'-Nitroflavone(23) | 201-3° Dioxan | — | — | — | — | — | b |
| 3 | 4'-Nitroflavone | 244-6° Glacial acetic acid and acetone-dioxan | C ₁₅ H ₉ O ₄ N Colourless crystals | C H N | 67.0 3.6 4.5 | 67.4 3.4 5.2 | 80 | a |
| 4 | 3', 5'-Dinitroflavone | 297-9° Acetic acid and dioxan | C ₁₅ H ₇ O ₆ N ₂ Colourless crystals | C H N | 57.4 2.7 9.2 | 57.7 2.6 9.0 | — | a, c |
| 5 | 2-Styrylchromone ² | 138-40° Alcohol | — | — | — | — | — | a |
| 6 | 2-(<i>m</i> -Nitrostyryl)-chromone | 233-5° Dioxan | C ₁₇ H ₁₁ O ₄ N Pale yellow crystals | C H N | 69.3 3.7 5.2 | 69.6 3.8 4.8 | 56 | a |
| 7 | 6-Methoxyflavone(12) | 162-3° Aqueous alcohol | — | — | — | — | 70 | a |
| 8 | 4'-Nitro-6-methoxy-flavone | 205-6° Dioxan-water | C ₁₆ H ₁₁ O ₅ N. H ₂ O Pale yellow crystals | C H N H ₂ O ³ | 61.4 4.0 4.5 5.5 | 61.0 4.1 4.4 5.7 | 60 | a |
| 9 | 6-Methoxy-2-styryl-chromone | 145-6° Aqueous alcohol and ligrom | C ₁₈ H ₁₄ O ₃ Colourless needles | C H | 77.1 5.2 | 77.7 5.0 | 80 | a |

¹ Methods of cyclization.—

(a) Glacial acetic acid with a trace of mineral acid at 100°

(b) Heating above m.p.

(c) The diketone m.p. 227-8°, was heated in a silicone bath (b.p. 300°), when formation of the flavone (m.p. 296-300° from dioxan), occurred with solidification.

² Cheema, Gulati and Venkataraman (8) give m.p. 131°

³ Loss in weight at 100° when heated under reduced pressure for one hour.

Preparation of flavone from o-hydroxyacetophenone.

A mixture of *o*-hydroxyacetophenone (6.8 g.), benzoyl chloride (7.0 g.) and pyridine (10 ml.), which had been heated (calcium chloride guard tube) at 100° for 15 min. (See (1)) was poured into dilute hydrochloric acid (1%; 300 ml.), and the solid product was washed and sucked at the pump for 5 min. The crude ester was dissolved in pyridine (30 ml.), and the solution treated at 50° with potassium hydroxide (2.5 g.), which had been pulverized in a hot mortar. The mixture was agitated for 5 min., during which time a copious precipitate of the yellow potassium salt of *o*-hydroxydibenzoylmethane was formed. Excess of aqueous acetic acid (10%) was then added, and the precipitated diketone was freed from adhering liquid at the pump. It was dissolved in concentrated sulphuric acid (70 ml.) and after 15 min. the acid solution was poured into crushed ice. The precipitated flavone (4.7 g. : 42% yield) had m.p. 92–3°, mixed m.p. with an authentic specimen (m.p. 97°), 93–5°. The yield of crude ester from *o*-hydroxyacetophenone was 70%, of crude diketone from crude ester 78%, and of flavone from crude diketone 77%.

Preparation of 3':5'-Dinitro-6-methoxyflavone (XIV) from 2-(3':5'-dinitrobenzoyloxy)-5-methoxyacetophenone.

The sodium salt of 2-hydroxy-5-methoxyacetophenone (X, 2.5 g.) was added to a solution of 2-(3':5'-dinitrobenzoyloxy)-5-methoxyacetophenone (4 g.) in pyridine (20 ml.), and after it had been kept for three days, the mixture was acidified with dilute hydrochloric acid (10%). The brown solid thus obtained—the chloroform solution of which was not extracted with aqueous sodium hydroxide (2%) and was, therefore, not the diketone—separated from glacial acetic acid in pale yellow needles, (2 g.) m.p. 301–3°, which gave a pale green fluorescence with sulphuric acid. (Found; C, 56.4; H, 3.3; N, 7.9. $C_{16}H_{10}O_7N_2$ requires C, 56.1; H, 2.9; N, 8.2%).

Preparation of 3':5'-Diaminoflavone.

A suspension of 3':5'-dinitroflavone (2.5 g.) in hot concentrated hydrochloric acid (45 ml.) was treated with stannous chloride (11 g.), and the white precipitate, which replaced the original flavone, was separated after the mixture had been kept at 0° C. for some time, washed with concentrated hydrochloric acid and dissolved in water. The resulting solution was made strongly alkaline with aqueous sodium hydroxide (20%), and the free base thus obtained crystallised from alcohol, from which it separated in yellow crystals having a m.p. 237°. Yield, 0.5 g. Found: C, 71.4; H, 4.9, N, 10.8. $C_{15}H_{12}O_2N_2$ requires C, 71.3; H, 4.8; N, 11.1%).

The diacetyl derivative (pyridine-acetic anhydride) had m.p. above 350° on recrystallisation from glacial acetic acid (Found C, 67.9, H, 4.8; N, 8.3 $C_{19}H_{16}O_4N_2$ requires C, 67.9; N, 4.8; N, 8.3%).

SUMMARY

A variety of salts of weak acids, *e.g.* sodium hydroxide, phenoxide, peroxide, hydride, ethyl sodioacetoacetate, triphenylmethylsodium, potassium acetate and benzoate have been found to be effective reagents for the transformation of *o*-aroyloxyacetarones into the corresponding *o*-hydroxydiaroylmethanes, which can be

cyclized to the corresponding chromones. The results obtained indicate that the reaction involves a base-catalysed intramolecular condensation of the Claisen type. The effect of nitro- and methoxyl groups on the transformation is discussed, and the preparation of some new flavones is described.

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DEPARTMENT OF CHEMISTRY,
UNIVERSITY COLLEGE,
DUBLIN.

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SEED TRANSMISSION OF VIRUS YELLOWS OF SUGAR BEET (*Beta vulgaris* L.) AND THE EXISTENCE OF STRAINS OF THIS VIRUS IN ÉIRE

By

PHYLLIS E. M. CLINCH and J. B. LOUGHNANE.

(Department of Plant Pathology, University College, Dublin).

(PLATES 13-17)

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INTRODUCTION.

SUGAR beet is a comparatively new crop in Éire and it was not until recent years that abnormal yellowing of the beet foliage became a matter of grave concern to the Irish Sugar Company's Agricultural Advisers. From the descriptions of Dutch and English workers (11) (15) (8) it was apparent to them that much of this yellowing was probably of virus nature, corresponding with the disease known as "vergelingsziekte" in Holland, "jaunisse" in Belgium, and "virus yellows" in England. However, the situation was complicated by the existence of other factors liable to induce yellowing in beet, such as Downy Mildew (*Perenospora Schachtii*) and certain mineral deficiencies in the soil. Yellowing was particularly widespread in 1946 and in August of that year it was arranged that affected plants should be examined in this laboratory in order to identify with certainty diseases of virus origin. Dr. M. A. Watson, of Rothamstead Experimental Station, visited Éire in October, 1946, and expressed the opinion that most of the yellowing present in the field crops (100% in certain cases), was due to virus infection.

Particular interest was centred on a yellowing which occurred in a new variety (family 41) of sugar beet bred by Mr. B. Crombie at the Sugar Company's Station at Mallow, Co. Cork. The cross had been made in a pollen-proof cage in 1945, the parent plants having been selected from commercial crops. In 1946 three separate crops from the resulting seed showed, a few weeks after singling, approximately 25% of yellowing which, according to Mr. Crombie, resembled typical virus yellows, but did not appear to spread to neighbouring beet crops. The manner of its occurrence suggested that this form of yellows was seed-borne. However, Quanjer (11) who had studied virus yellows for a considerable period, found no evidence that the disease was transmitted through the seed; and although published accounts of tests with seed from diseased plants are scarce, it appears to be generally accepted that virus yellows is not seed-borne. It was, therefore, tentatively assumed that the

yellowing in family 41 was probably not of virus nature, but was a variegation resulting from a genetical abnormality. Transmission tests in this laboratory subsequently proved this assumption to be incorrect, and a note to this effect has already been published (5). A more detailed account of the experiments with family 41 and with specimens from commercial beet crops is now submitted

MATERIALS AND METHODS.

Healthy and affected field-grown plants of family 41, together with a small quantity of the residual seed of that family, were received from the Sugar Company on 7th September, 1946. For the testing of commercial crops specimens of sugar beet and mangold plants showing virus-like symptoms of the type described by Hull and Watson (8) were selected from field crops in Dublin and Carlow and re-planted in pots in a glasshouse at the Albert Agricultural College, Glasnevin. The specimens were full-grown plants all of which showed the typical thickening and yellowing of the lower leaves. A small number of the sugar beet plants showed, in addition, mottling and more or less crinkling of the leaves and were selected as possible examples of mixed infections with the yellows and mosaic viruses.

The virus content of each specimen was determined by carrying out transmission tests from the affected plants to healthy plants, using as vectors the aphides *Myzus persicae* (Sulz.) and *Aphis Fabae*, both of which were shown by Roland (11) and van Schreven (13) to transmit yellows and mosaic viruses of sugar beet. Sap inoculations using carborundum powder were also performed with a view to separating the mosaic virus, if present, from yellows. Sugar beet of a commercial variety (No. 347) obtained from Mr. Crombie, and spinach (*Spinacia oleracea* L.), were used as experimental hosts. The seed was usually sown in 10-inch pots and thinned to 7-10 seedlings which were submitted to infection when the first pair of true leaves were about 3 inches in length. Various larger sizes were also experimented with, including well-developed plants each in a 7-inch pot. Susceptibility did not appear to vary to any appreciable extent with size, provided the plants were in an active state of growth during the experimental period.

The stock of *Myzus persicae* used in the experiments was reared on turnip and cabbage plants; *Aphis Fabae* was collected from the spindle tree (*Euonymus*), and later reared on beans. The transmission tests were carried out in a glasshouse in insect-proof cages constructed of timber with two sides of very fine mesh copper gauze, the top and remaining sides being of glass. The bottom of each cage consisted of perforated zinc which was covered by two 3-inch layers of gravel and fine sand, respectively. The plant to be tested was placed in a cage and colonised with the appropriate vector. When the insects had multiplied sufficiently, the pots containing the experimental seedlings were included in the cage and allowed to remain for 2-7 days. The longer period was allowed in the earlier experiments in order to ensure considerable movement of insects from the source to the healthy plants. At the end of the feeding period the plants were fumigated with nicotine vapour and moved to another glasshouse for observation. When necessary the seedlings in 10-inch

pots were later transplanted separately to 7-inch pots thus permitting an extended observation period. Transplants were also made to outdoor plots in order to observe symptoms under field conditions.

INVESTIGATION OF YELLOWING IN FAMILY 41.

A small portion of the seed of this family was sown in September 1946 and the remainder on 10th March, 1947, both lots being kept in separate insect-proof glass-houses. The Autumn sown seedlings were transplanted to 7-inch pots when of appropriate size and kept over Winter in an unheated glasshouse. During the dormant period they already had 2-4 true leaves approximately 5 inches in length, but these showed no signs of virus infection. Owing to severe weather active growth was not resumed until the last week of March, 1947. On 22nd April, the first signs of yellowing were observed at the tips of the lower leaves of 2 plants. During the following 3 weeks further plants of the batch showed similar symptoms. In all, 11 out of 33 plants were affected. The seedlings from the March sown seed received no check in growth. They were transplanted to 7-inch pots when the first pair of true leaves were about 3 inches in length and proceeded to grow vigorously. Only one plant appeared stunted and by 16th May, the two basal leaves of this plant were distinctly yellow at the tips, had ceased to expand and were tough and leathery in texture. All the remaining plants appeared healthy until they were about 10 inches in height with 10-12 foliage leaves. Then the tips of the lower leaves of some of them began to thicken and turn pale, eventually becoming yellow or orange in colour. Between May 17th and June 7th, 57 of the 110 plants developed pathological symptoms. Where two or three plants derived from an individual cluster, there was no uniformity in the number showing yellowing. One or both of a pair or two out of three might be affected; on the other hand a single cluster might give rise only to healthy plants. However, the majority of the seedlings raised were from separate clusters.

The yellowing usually spread uniformly from the tips to the proximal ends of the leaves, only the areas around the veins and the basal portion at the junction of lamina and petiole remaining green (Fig. 1). Sometimes the spread was asymmetrical, one side of a leaf being more extensively affected than the other (Fig. 3.). Occasional leaves showed yellowing at the tip (Fig. 2) sometimes accompanied by isolated patches of affected tissue elsewhere on the lamina which frequently were situated at the junction of two main veins. Thickening of any portion of the leaf was inevitably followed by yellowing. There was no discernible abnormality in the non-yellowed areas of affected leaves. Eventually the chlorotic areas withered and turned brown and the margins of the leaves curled upwards (Fig. 4). Various saprophytic moulds developed on the dead areas. Taking into account the number of infections in the Autumn sowing, the percentage of infected plants from the 143 seed clusters sown was 47.5.

The symptoms described above are essentially similar to those of virus yellows of sugar beet as described by Quanjer (10) Roland (11), and Watson (15). Demarcation of yellow from green areas by the veins, as seen in family 41, was described

in the case of virus yellows by Klinkenberg (9). Watson (15) (Plate 12, Fig. 4) and Hale, Watson and Hull (7) (Plate 1, Fig. 5) illustrate this condition in leaves from field plants, but considered this localization to be the result of late infection of well developed plants.

Transmission Tests with plants of Family 41 showing Yellowing.

It has already been reported (5) that transmission of the yellows disease in family 41 to healthy sugar beet and spinach seedlings was obtained in three different experiments using *Myzus persicae* as vector. Of the 68 sugar beet and 34 spinach plants submitted to infection, 85% and 76%, respectively, developed yellowing. In three further experiments performed during August and September, 1947 and using different plants of family 41 as sources, similar results were obtained. No infection was secured in three experiments carried out during June, July and August, using *Aphis Fabae* as vector and involving 48 sugar beet seedlings as experimental hosts. In parallel experiments with mild virus yellows, *A. Fabae* transmitted the disease to only 2 of the 48 experimental plants.

The symptoms in the experimental plants began to appear 3-4 weeks after infection when the tips of the lower leaves became tough and leathery and then gradually turned yellow. There was no vein clearing and the ensuing disease was essentially similar to a mild virus yellows isolated from commercial crops which is described in another section of this paper. The affected plants were only slightly stunted compared with healthy ones. As the symptoms were more or less confined to the lower leaves, they showed up best under conditions favouring the growth of sugar beet which in the glasshouse at Glasnevin are those prevailing during the Spring and Autumn months. Hot, sunny weather was unsuitable for the display of symptoms under glass as the plants tended to wilt and the lower leaves to wither. On the other hand, symptoms of yellowing were repressed if the plants were grown in a very dull light, due presumably to the lowered photosynthetic activity and consequent reduction in the level of surplus carbohydrate. This is true of mild virus yellows isolated from field crops as well as of the disease transmitted from family 41. Plants grown out-of-doors showed consistently good symptoms during the season.

The foregoing results proved conclusively that the yellowing in family 41 was due to a seed-borne virus, and it is assumed that one, or both, of the parents of this family was infected with virus yellows. As seed transmission of this virus has not hitherto been observed, it must be concluded that virus yellows does not normally infect the embryo of the sugar beet seed. Indeed there is no information as to whether it occurs in any part of the seed. Bennett and Esau (2) have shown that the Curly Top virus of sugar beet reaches a very high concentration in the mature seed, but that the actual embryo appears never to become infected. The fact that virus yellows was apparently able to penetrate with ease into the embryo in the case of family 41 suggests that a genetical abnormality in this family may possibly have affected the permeability of certain cells of the embryo thus permitting diffusion of virus into regions where it does not normally penetrate. The behaviour of the seed in subsequent generations will probably throw some light on this subject.

Accumulation of Starch in Foliage of Family 41.

Quanjer (10) showed that starch accumulated in the yellowed leaves of virus-infected sugar beet plants and both Quanjer and van Riemsdijk (12) concluded that this resulted from inhibited translocation rather than from a disturbance in the respiratory mechanism. Tests for starch accumulation in affected leaves of family 41 were made by removing potted plants from the greenhouse at 5 p.m. on days of bright sunshine and placing them in a dark room for periods varying from 48 to 72 hours, after which appropriate leaves were cut from the plants, dipped in boiling water and decolorised by soaking in warm alcohol. They were then stained in a dilute solution of iodine. The information derived from several such tests performed during the months of April, May and June was as follows: After 48–72 hours in darkness no starch is demonstrable in the leaves of healthy plants nor in the leaves of affected plants which are of normal colour and texture. Starch accumulation is obvious in the toughened, but still green, areas of affected leaves, but is most conspicuous in the yellowed areas. The demarcation of affected and normal areas by the main veins in some leaves is striking (Figs. 5 and 6). Isolated yellow spots appear as dark blue patches on the decolorised and stained leaves. Examination of the lower leaves of old plants showed that prior to the onset of necrosis the starch begins to disappear from the yellow areas, being probably used in respiration. The regions which are first to become yellow are also those from which the accumulated starch is first to disappear for when old leaves showing general yellowing were stained with iodine after a period in darkness, only the interveinal areas of the distal portion of the leaf failed to turn blue. Staining of leaves of similar age showing yellowing only in the distal half, also revealed the disappearance of starch from the strongly chlorotic tip and accumulation of starch in the remainder of the yellow area. It was noteworthy in the case of these mature leaves that the green areas of those showing only partial yellowing were functioning normally in regard to translocation, being devoid of starch following a sojourn in darkness.

Hand sections of affected leaves of family 41 cut during the afternoon on days of bright sunshine showed the chloroplasts of the soft, green areas to be normal in appearance and indistinguishable from those of healthy leaves. In areas which were still green, but which had developed the toughened texture which precedes yellowing, the chloroplasts were gorged with starch, and this was also the situation in the yellow areas in the earlier stages. In the completely yellow or whitish areas of leaves affected for some time only the remains of degenerated chloroplasts were present. These are similar to the effects observed by Klinkenberg (9) in sections of leaves affected by virus yellows.

INVESTIGATION OF YELLOWING IN SUGAR BEET AND MANGOLD PLANTS SELECTED FROM FIELD CROPS.

All the plants selected from commercial crops as probable sources of virus were found to be infected, and a high percentage of infection in the experimental hosts was readily secured using *Myzus persicae* as vector. Repeated sap inoculations

from the various sources, however, with and without the use of carborundum powder, failed to produce any disease symptoms.

In all cases, including those in which the sugar beet sources had shown more or less crinkling and mottling, the disease transmitted by the insect vector was of the yellows type. There was no evidence of the presence of a mosaic virus in the specimens examined, and the mottling observed in some of them at pulling time was obviously not the result of virus infection. Two types of yellows disease were, however, isolated, which are believed to be strains of the same virus and which for convenience may be designated as mild yellows and etch yellows, respectively.

Mild Yellows—The first symptoms of infection in rapidly growing seedlings of sugar beet and spinach appear about 18–21 days after inoculation and consist in a toughening of the tips of the lower pair of leaves which then lighten in colour and gradually turn yellow. The yellowing usually spreads downwards through these leaves, only the areas around the veins remaining green, and the texture of the leaves becomes thickened. Similar effects meanwhile appear in the next leaves above, so that the symptoms develop acropetally but do not advance beyond the medium sized leaves and are frequently confined to the lower ones. Later, the chlorotic areas of the older affected leaves become brown and necrotic and the entire lamina becomes dry and brittle and tends to curl upwards at the margins, especially in glasshouse plants. Growth of the infected plants is somewhat stunted compared with that of healthy plants.

Etch Yellows.—Under optimum conditions symptoms develop in sugar beet and spinach 12–14 days after infection. The veins of the young leaves appear translucent when viewed by transmitted light and later may become whitish or necrotic and appear as if “etched” on the leaf (Figs. 7 and 8). The vein clearing appears first at the tip of the leaf, then all over the lamina, or it may spread asymetrically. About 16–21 days after infestation symptoms of yellowing develop independently of the etching in the lower leaves of the plant, in exactly the same manner as described for mild yellows. The yellowing proceeds acropetally but more leaves on the plant become involved than in the case of mild yellows and the plant as a whole is much more stunted. The etching of the veins is a primary symptom and is not apparent in young leaves which develop subsequently. It may, however, appear in the heart leaves of plants which have overwintered in the glasshouse when these resume growth in Spring. As the “etched” leaves increase in size they also turn yellow and rarely attain normal dimensions. In the later stages they may appear extremely tough and leathery, with sunken collapsed veins. A common feature of the etch-infected plants, especially under glasshouse conditions, is the development of minute reddish rings or spots on some of the older yellowed leaves (Fig. 9). This symptom did not appear to be associated with mild yellows and is possibly a characteristic, if not a constant symptom of the etch strain. However, further observation of plants affected with both strains, and grown under varying environmental conditions, would be necessary in order to confirm this view.

As already mentioned, the mild and etch yellows are both freely transmissible by *Myzus persicae*. Etch yellows is the more infectious of the two and the rate of

infection with this strain was usually 100%. *Aphis Fabae* proved to be a poor and erratic vector, and in four different experiments in which this aphid was employed to transmit mild yellows, the respective numbers of sugar beet seedlings which became infected were 3/24, 4/36, 0/24, 0/12. A somewhat higher rate of infection was obtained in the case of etch yellows. Both strains can be transmitted by *Macrosiphum solanifolium*, but the actual efficiency of this aphid as a vector of yellows has not yet been determined.

It was considered desirable to test the possibility that the venal etching in "etch yellows" might be due to a contaminating virus in the original source of inoculum and not symptomatic of virus yellows. Accordingly, *Myzus persicae* was fed on (a) young leaves from the tops of sugar beet plants immediately after the development in them of vein clearing, and (b) lower leaves showing yellowing only. After 48 hours the insects were transferred to healthy sugar beet and spinach seedlings. In two such experiments it was found that vein clearing and subsequent yellowing developed in the test plants irrespective of whether the vectors had fed on sources (a) or (b). On the other hand, sap inoculations from similar leaves to healthy sugar beet and spinach seedlings failed to cause infection. Although these results do not eliminate the possibility that a mixture of viruses may be involved, they strongly support the view that the etching is merely a symptom of a particular strain of virus yellows and not that of a distinct virus.

The probable existence of strains of virus yellows was first suggested by Watson (14) on finding that the symptoms of the diseases which she isolated by insect from affected sugar beet specimens from Holland and from different counties in England differed in the intensity of their symptoms. The more severe strains were said to produce a vein clearing of the medium aged leaves (never the very youngest leaves), followed later by the appearance of local symptoms on the older leaves. No further references to the existence of strains of virus yellows appear to have been made, and in a recent paper by Hale, Watson and Hull (7) "clearing" and "etching" of the veins was described as a symptom of virus yellows associated with early infection of field plants. It was further stated by these authors that as the "etched" leaves develop they become yellowed so that in such plants the typical "yellows" symptoms occur first on leaves of medium age. In the present work it was found that the onset of yellowing occurs independently of the etching and that the latter is a primary symptom of what is apparently a particular strain of virus yellows and not simply the resultant of infection at a certain stage of development of the plant. The vein clearing appeared consistently, irrespective of whether the hosts were very young seedlings or well developed plants at the time of infection. It is also a persistent character having appeared in successive transfers from September 1946 to the end of the 1947 season.

Introduction of Etch Yellows to plants already infected with Mild Yellows.

Six plants of family 41, three infected with mild yellows and three apparently healthy, were colonised with viruliferous aphides (*M. persicae*) from a sugar beet plant infected with etch yellows. In due course all six plants developed vein clearing

in the young leaves showing that entry of the etch virus had not been prevented by the previous presence of the mild yellows. Following this a similar test was made using plants of the commercial variety No. 347 infected with the mild strain of yellows which had been isolated from various field plants. The result was the same as in the case of family 41, the experimental plants showing etching in the young leaves about 14 days after infection.

It has been established in the case of various sap-transmissible viruses, such as tobacco mosaic and potato virus X, that a plant infected with one strain cannot be infected by sap inoculation with another strain of the same virus. The evidence in regard to this cross immunity between strains has been summarised by Bawden (1). It indicates clearly that the "protection" is not due to inactivation of the one strain by the other but seems to depend entirely upon the inability of one strain to multiply in cells already occupied by another strain of the same virus. In the case of potato virus X, for example, it has been demonstrated that the protection is absolute in the parenchymatous tissues of the leaves and superficial layers of the stem of the potato. In such tissues the virus is believed to be generally distributed and present in high concentration. Complete protection, however, is obviously not afforded to the internal tissues of the stem for a second strain of X can be introduced to a plant already infected with this virus by means of a graft to either the aerial stem or tuber (3) (4). This is not due to absence of the "protecting" virus from the phloem, for it is known that virus X moves through the phloem although it may not multiply therein (3). It might be due to a lower concentration of virus in stem tissues. The authors are not aware of any experiments dealing with cross immunity between strains of insect-transmissible viruses in which the second strain is introduced (usually direct to the phloem) by means of the insect vector. The evidence in the case of virus X suggests strongly that there might not be any barrier to the entry of the second virus in these circumstances. Consequently, the ability of the etch virus to infect plants already containing a mild strain of virus yellows must not be taken as proof that the former is unrelated to the latter.

Internal Pathological Changes induced by Etch Yellows.

Quanjer (10) in 1934 was the first to draw attention to internal pathological changes associated with the yellows disease of sugar beet. He observed that in the secondary phloem of the yellowing leaves certain groups of sieve tubes and companion cells became filled with a yellow "gum" occupying the entire lumina of the cells, the walls of which also become yellow and sometimes swell. He referred to this condition as "gummosis" and contrasted it with the effects of the Curly Top disease of sugar beet in which hypertrophy and hyperplasia occur in the pericycle and phloem followed by the exudation from these cells of a viscid liquid as well as by extensive necrosis. From Quanjer's description of the symptoms it would appear that the strain of yellows with which he worked was of the mild type.

Watson (14) in 1940 reported failure to find gummosis in the phloem of glass-house plants, even in very well-established infections. Klinkenberg (9), however, confirmed that gummosis is a constant feature of virus yellows but stated that it

develops only after the flow of carbohydrates has been arrested and should therefore be regarded as a secondary effect of the pathological condition of the phloem, rather than as the primary cause of inhibited translocation. She suggests, however, that there is probably a disturbance in the phloem prior to the appearance of its visible evidence in the form of gummosis.

Examination of leaves from sugar beet affected with etch yellows affords evidence that there is a direct effect on the phloem following infection with this virus. Sections were prepared from (a) a young tip leaf approximately 3 cms. in length and 1 cm. in width, 4 days after the development in it of vein-clearing and previous to the appearance of yellowing in the lower leaves of the plant, and (b) an upper leaf showing vein-clearing from a plant also showing yellowing in the lower leaves. The material was fixed in Regaud's fluid and embedded in paraffin. Sections were cut at 8μ and stained in Heidenhain's iron alum haematoxylin and counterstained with erythrosin. Sections from leaf (a) showed that one of the initial symptoms of infection is hypertrophy of the parenchyma cells adjoining the phloem (Fig. 10), and also necrosis of the sieve tubes and hypertrophy and hyperplasia of the adjacent parenchymatous tissues as shown in Figs. 11, 12 and 13. These effects were visible in many veins of the leaf. Fig. 14 represents a single vascular bundle from the midrib of leaf (b) showing phloem necrosis and disruption of the bundle cap at one side. Several sections from leaf (b) showed complete collapse of the parenchymatous cells adjoining the phloem tissue of the vascular strands (Fig. 15), which is obviously the condition underlying the severely "etched" appearance of the veins. The translucent effect would result from the hypertrophy and disintegration of chloroplasts in the mesophyll tissues seen in sections of leaf (a).

These effects resemble the primary effects of Curly Top of Sugar beet described by Esau (6). However, in the latter disease the necrosis is more severe and is usually succeeded by extensive proliferation and radial elongation of parenchyma cells external to the vascular bundles, resulting in the thickened veins and overgrowths which are a characteristic macroscopic symptom of the disease. There is also a conspicuous amount of phloem exudate. The work of Bennett and Esau (2) has shown that the Curly Top virus exists and multiplies probably exclusively in the phloem of affected host plants. In sugar beet affected by etch yellows, the above mentioned pathological changes, of which vein clearing is the external sign, are ephemeral. Their development, however, shows that a disturbance in the phloem is one of the earliest effects of the virus. Considering the nature of the subsequent symptoms of the disease, which Quanjer (10) and van Riemsdijk (12) have shown to be due to inhibited translocation, it seems probable that this phloem disturbance exists permanently in the affected plants, even though further visible changes in the phloem tissues, such as gummosis, may not take place until after the development of yellowing in the lower leaves.

ETCH YELLOWS IN WILD BEET (*Beta maritima*).

Roland (11) was the first to observe that virus yellows overwinters in the roots of *Beta maritima* and other workers have confirmed this observation. In 1947 Mr. B.

Crombie observed yellowing in wild beet growing in Co. Kerry in the extreme south-west of Éire. Specimens were forwarded to this laboratory for examination, but an immediate transmission test was impracticable as the plants withered completely after having been potted in the glasshouse. They were placed out-of-doors to recover and in due course made luxuriant growth, the lower leaves showing yellowing as before. Transmission tests from these plants to sugar beet and spinach, using *M. persicae* as vector, resulted in typical etch yellows symptoms in the host plants. Whether the etch yellows was present in the plants when originally received is not certain, as the infection might possibly have been contracted during the period mentioned above when the plants were out-of-doors. It is clear, however, that *Beta maritima* is susceptible to etch yellows.

ATTEMPTS TO INFECT SOLANACEOUS HOSTS WITH VIRUS YELLOWS.

Owing to the similarity in the physiological effects on the host plant of virus yellows and the leaf roll virus of potato, tests were made to see if potatoes could be infected with the sugar beet virus. For this purpose six sprouting tubers of the variety Arran Cairn and six of Dunbar Standard (both of which are very susceptible to leaf roll), were colonised with *Myzus persicae* from a sugar beet plant affected with etch yellows. A similar number of tubers was colonised with insects from a yellowed plant of family 41. After some days the insects were removed and the tubers grown on in the glasshouse. None of the resulting plants showed symptoms. Similar tests with *Nicotiana glutinosa*, *Nicotiana rustica* and *Datura stramonium* also gave negative results.

ACKNOWLEDGMENTS.

The authors are greatly indebted to Professor R. McKay for his interest and helpfulness during the course of this investigation. They also wish to thank Mr. B. Crombie and other officials of the Irish Sugar Company who furnished them with specimens and information regarding the beet crops. The photographs were taken by Mr. G. H. McLean, A.R.P.S.

SUMMARY.

Seed of a new family of sugar beet (No. 41), bred in Éire in 1945, when sown in an insect-proof glasshouse gave rise to plants a high percentage of which showed a yellows disease indistinguishable from virus yellows isolated from field crops of beet and mangold.

The disease was non-transmissible by sap inoculation to healthy sugar beet and spinach plants. Transmission was successfully accomplished in 6 different experiments using the aphid *Myzus persicae* (Sulz.) as vector. In 3 different experiments *Aphis Fabae* failed to transmit the virus.

The physiological effects of the disease in family 41 are similar to those of virus yellows. Starch accumulates in the toughened and yellow areas of affected leaves and is retained therein after the plants have been 48-72 hours in darkness.

Two types of yellows disease were isolated from commercial sugar beet crops in Éire. These are designated mild yellows and etch yellows, respectively.

In the case of mild yellows, the first symptoms to appear are a toughening and yellowing of the basal leaves. The yellowing spreads acropetally in the lower parts of the plant, the chlorotic areas subsequently becoming necrotic. The primary symptom of etch yellows consists of a clearing of the veins of the young developing leaves which become whitish or superficially necrotic. Subsequently the symptoms are similar to those of mild yellows, except that the chlorosis is more extensive and growth of the plant is more stunted. Minute reddish rings or spots which develop on the yellowed leaves are believed to be characteristic of the disease.

Both diseases are readily transmitted by *Myzus persicae*, but *Aphis fabae* proved to be a poor and erratic vector. They are also transmitted by *Macrosiphum solanifolii*, but the comparative efficiency of this aphid as a vector of yellows has not been estimated.

No evidence was obtained that two different viruses were responsible for the vein clearing and yellowing symptoms in etch yellows. Although etch yellows can infect plants already containing mild yellows, the former is, nevertheless, regarded as a strain of the latter.

Examination of the internal anatomy of young sugar beet leaves showing vein clearing following infection with etch yellows revealed that the phloem tissues become necrotic and there is hypertrophy and hyperplasia of the surrounding parenchymatous cells. The latter may eventually collapse.

The wild beet (*Beta maritima*) is susceptible to etch yellows. Various solanaceous plants, including the potato (*Solanum tuberosum*) proved to be non-susceptible to etch and mild yellows.

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- (15) ———— - Ans. App. Biol., **29**: 358-365, 1942.

EXPLANATION OF PLATES.

PLATE 13

- Fig. 1. Lower leaf from sugar beet plant of family 41 showing general yellowing due to seed-borne virus.
- Fig. 2. Leaf from affected plant of family 41 showing yellowing confined to tip. Note sharp demarcation of yellow and green areas.

PLATE 14.

- Fig. 3. Leaf from affected plant of family 41 showing asymmetrical development of yellowing.
- Fig. 4. Lower leaf from plant of family 41 showing final stage of disease.

PLATE 15.

- Figs. 5 and 6. Lower leaves from affected plants of family 41 which had been kept in darkness for 48 hours. Decolorised and stained with iodine. Showing retention of starch in areas which had shown yellowing in fresh leaf.

PLATE 16.

- Fig. 7. Upper leaf from young sugar beet plant showing conspicuous vein clearing following infection with etch yellows.
- Fig. 8. "Etching" of veins in young leaf of spinach (*Spinacia oleraceae*) following infection with etch yellows.
- Fig. 9. Lower leaf of sugar beet plant affected with etch yellows showing numerous minute rings and spots. These were reddish in colour.

PLATE 17.

Photomicrographs of sections of young leaves of sugar beet showing vein clearing following infection with etch yellows. Stained Heidenhain's iron alum haematoxylin and erythrosin.

- Fig. 10. Hypertrophy of parenchymatous tissues adjoining phloem. This effect underlies the translucence or "clearing" of veins. X 258.
- Figs. 11 and 12. Necrosis in phloem and hypertrophy and hyperplasia of adjoining parenchymatous cells. X 245 and 260, respectively.
- Fig. 13. Transverse section through tip of leaf under low power showing necrosis in phloem and extending to surface. X 70.
- Fig. 14. Vascular bundle from midrib of leaf showing necrosis of phloem at one side of bundle cap and hypertrophy of adjoining parenchymatous tissue. X 254.
- Fig. 15. Section of leaf showing complete collapse of parenchymatous tissue adjoining the necrotic phloem. This effect gives rise to the severely "etched" appearance of the vein. X 178.

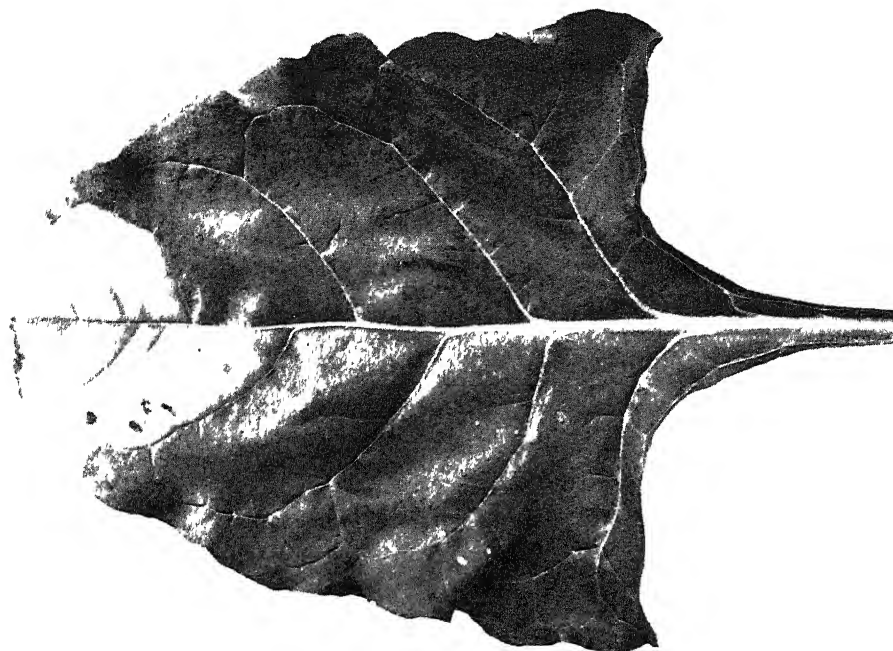


Fig. 2.



Fig 1.

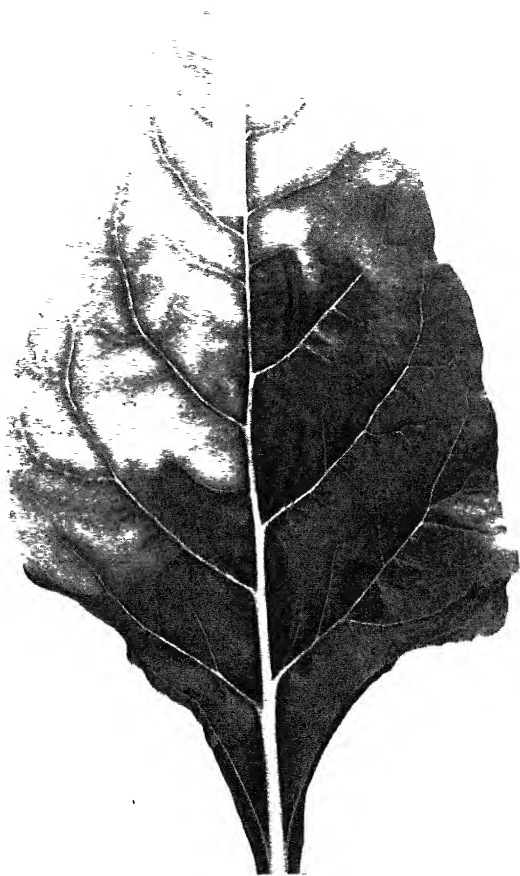


Fig 3

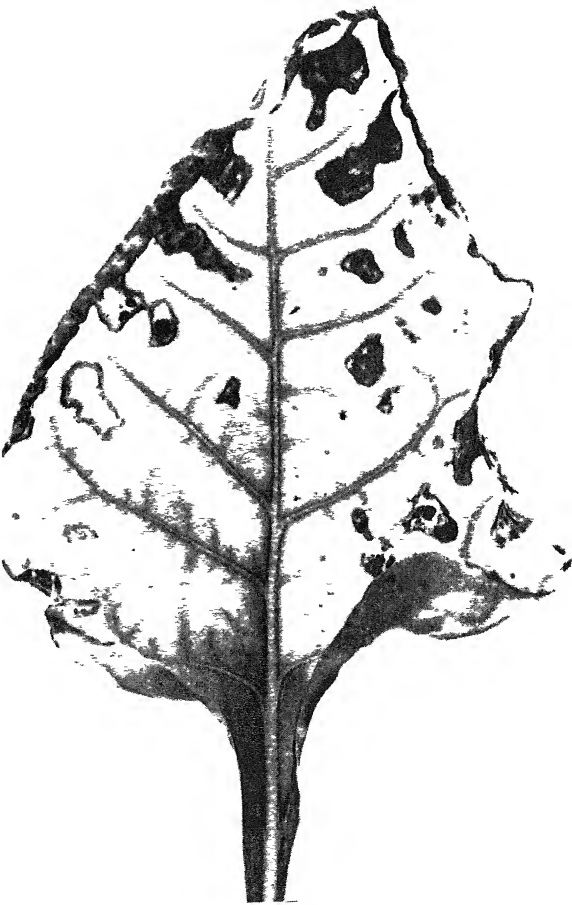


Fig. 4.

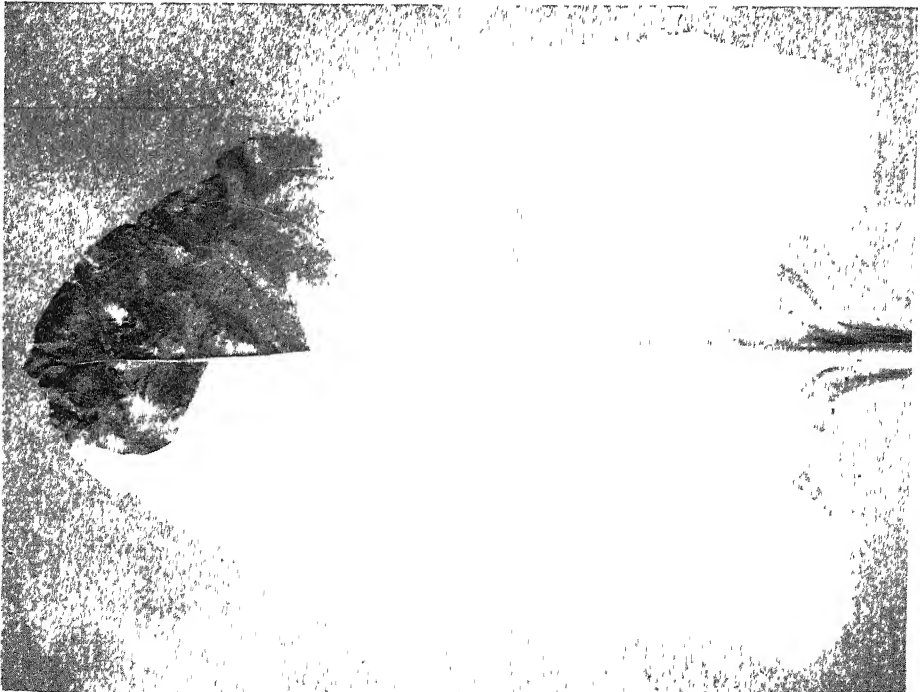


Fig. 5.



Fig. 6.

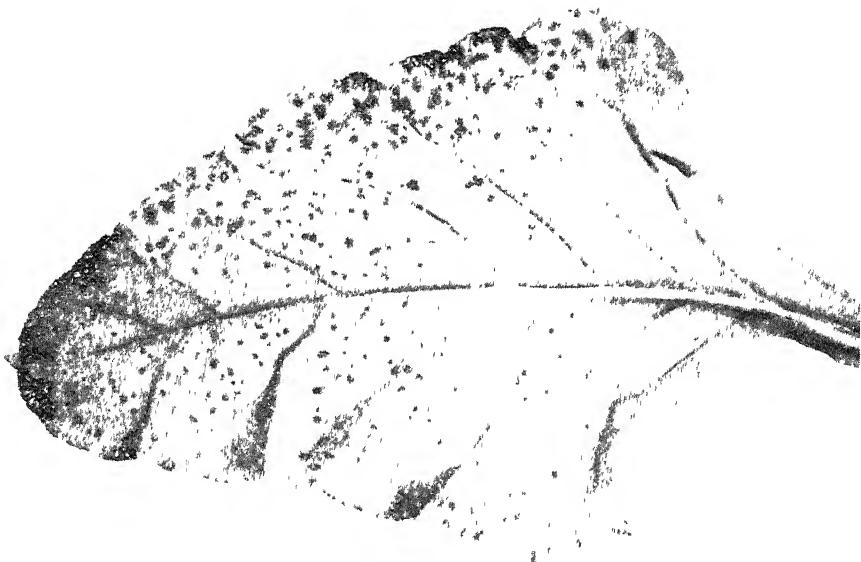


Fig 9

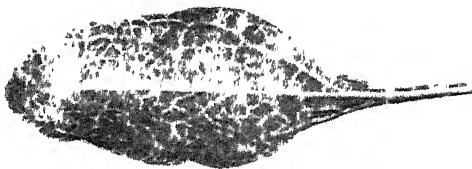


Fig 8

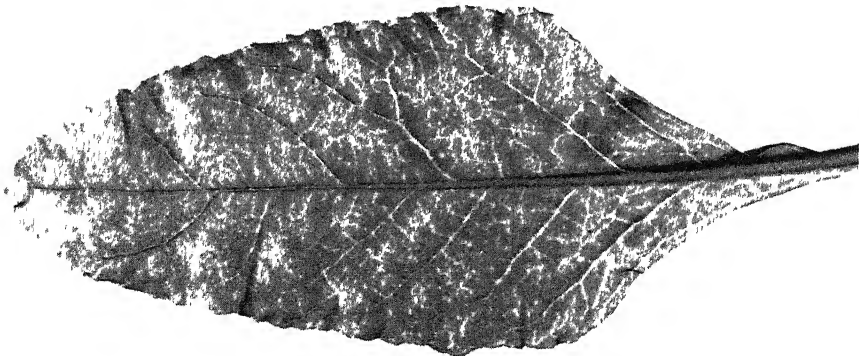


Fig. 7.

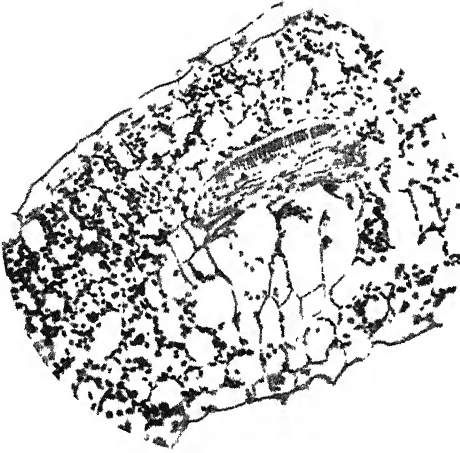


Fig. 10

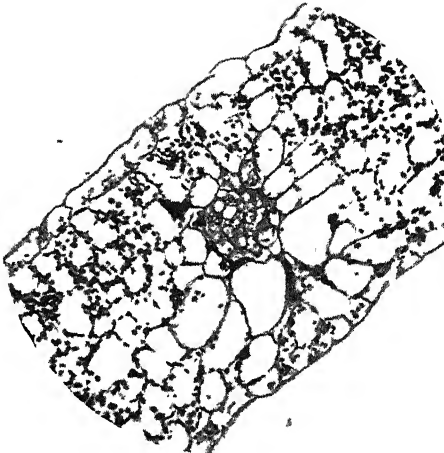


Fig. 11

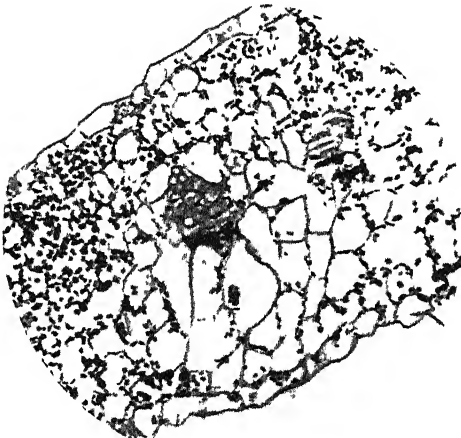


Fig. 12

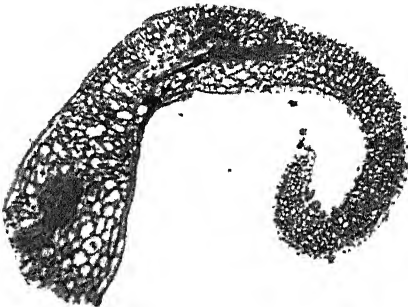


Fig. 13

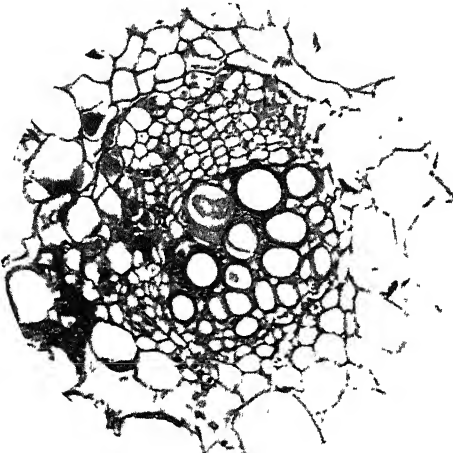


Fig. 14



Fig. 15

THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND.

BUELLIA CANESCENS PART 3.

THE CONSTITUTION OF DIPLOICIN.

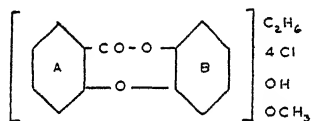
By

(The late) THOMAS J NOLAN, JOSEPH ALGAR, EUGENE P. McCANN,
WILLIAM A. MANAHAN AND NIALL NOLAN,

University College, Dublin.

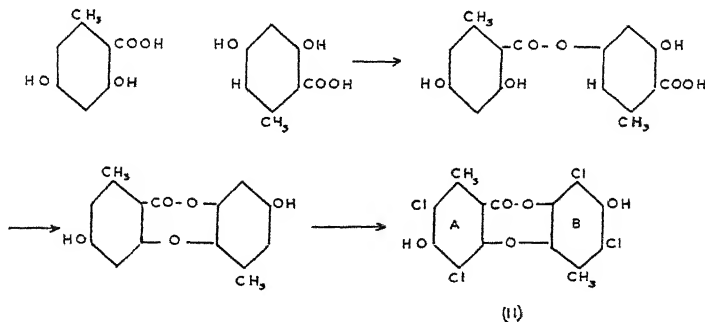
[Read MARCH 23. Published Separately, SEPTEMBER 6, 1948.]

IN Part 2 of this investigation it was shown by Spillane, Keane, and Nolan (Sci. Proc R D S 1936, **21**, p. 333) that the lichen *Buellia Canescens* contains, amongst other substances, the compound diploicin, to which they gave the formula $C_{16}H_{10}O_5Cl_4$. It was also shown by these workers that diploicin is a depsidone and that to it could be attributed the structure —

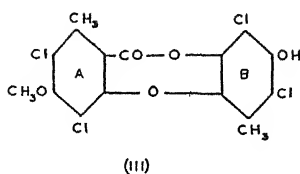


(I)

It was surmised that the C_2H_6 consisted of two methyl groups, rather than an ethyl group and a hydrogen atom, and that diploicin resulted from the condensation of two molecules of orsellinic acid, with subsequent elimination of one carboxyl group and simultaneous oxidation and chlorination. This would result in the compound II the monomethyl ether of which would be diploicin.

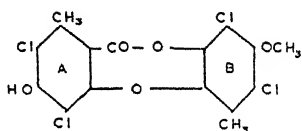


By analogy with known lichen substances it was regarded as most likely that the hydroxyl group in ring A had been methylated to form diploicin, which would accordingly have the structure III.



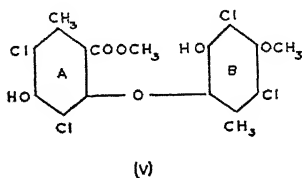
It should be noted that evidence so far given in proof of this configuration is limited to establishing the formula I, and that the complete formula III is based on analogy. A Kuhn-Roth determination of C-methyl has since been carried out and indicates the presence of two C-methyls, thus confirming the earlier surmise.

In the present communication, degradation experiments on diploicin are described which appear to prove that the structure of diploicin is correctly represented by the formula IV.



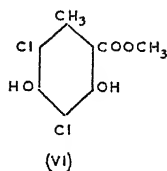
which places the methyl ether grouping in ring B and not in ring A, as was considered likely in the previously cited communication.

The orientation of the groups in ring A has been established in the following way. On treatment of diploicin with methyl alcoholic potash, the lactone bridge is opened to form the ester V (Spillane, Keane, and Nolan, *loc. cit.*).



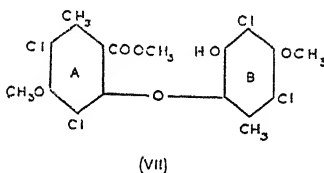
*2.4.3'.5'-tetrachlor—3.2'-dihydroxy—6-carbomethoxy—4'-methoxy—5.6'-dimethyl
diphenyl ether.*

The breakdown of the ester V by fission of the oxygen bridge was accomplished in the following way. The ester was subjected to super-chlorination, followed by reduction and hydrolysis. This resulted in the production of 4,6-dichlor-orsellinic acid methyl ester VI.



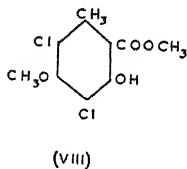
a substance which has been synthesised by Nolan and Murphy (Sci. Proc. R D.S. 1940, **22**, p. 317). Control experiments in the case of gangaleoidin (Davidson, Keane, and Nolan, Sci. Proc. R D.S. 1943, **23**, p. 143) had shown that super-chlorination does not result in demethylation, and consequently it was probable that the methoxyl group of diploicin, by inference, was in ring B. Definite proof of the occurrence of the free hydroxyl group in ring A was forthcoming from the following procedure.

Diploicin was methylated, and the lactone bridge then opened to form the ester VII (Spillane, Keane, and Nolan *loc. cit.*).



2.4.3'.5'-tetrachlor-2'-hydroxy-6-carbomethoxy-3.4'-dimethoxy-5.6'-dimethyl diphenyl-ether.

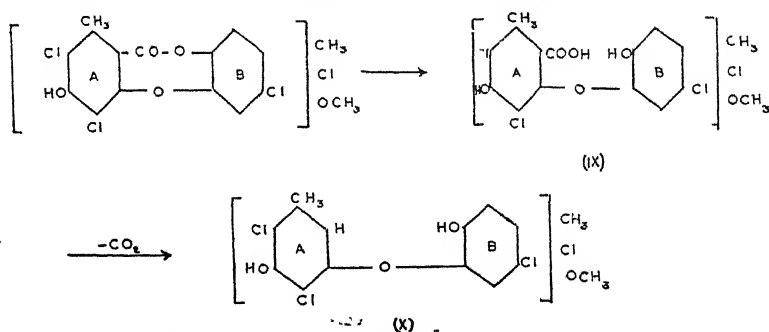
The ester VII was super-chlorinated, and on reduction and hydrolysis gave the mono-methyl ether of 4.6-dichlor-oriscellinic acid methyl ester VIII.



thus proving that the original methoxyl group in diploicin is in ring B and not in ring A. In the work so far described, no product was isolated which gave a clue to the orientation of the groups in ring B. It seemed likely that the most profitable method to isolate this half of the diploicin molecule, or a derivative thereof, would be to demethylate diploicin, and examine the nature of the product obtained, by submitting the resultant material to alkaline hydrolysis. Before doing so, the ester VII was examined for its colour reaction with 2,6-dichloroquinone-chlorimide in a sodium borate buffer solution. This reaction has been employed by Todd and Hems (J.C.S., 1940, p. 1208) as a test for a hydrogen atom para to a hydroxyl group in a benzene ring. Davidson, Keane, and Nolan (*loc. cit.*) have shown that it applies not only to hydrogen para to hydroxyl but also to certain negative substituents

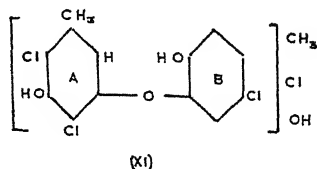
para to hydroxyl. Thus, para chlorphenol gives an intense blue, para hydroxy benzoic acid a deep blue, but para hydroxy benzoic ester gives no definite colour reaction. Now diploicin gives no definite colour reaction, but when the lactone bridge is opened to form the ester VII, thus setting free a hydroxyl group in ring B, a deep blue colour is obtained on testing. This colour reaction is therefore strong presumptive evidence for the presence of a chlorine atom in ring B in a position para to the oxygen atom of the lactone bridge (see IX).

When diploicin is subjected to alkaline hydrolysis and the resulting product is subjected to further hydrolysis or heated above its melting point, a series of compounds is obtained which may be explained in the following way:—

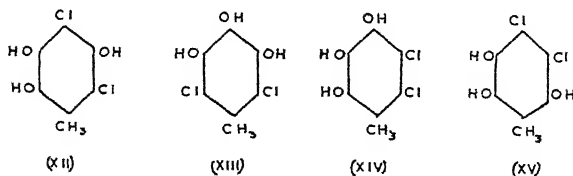


that is to say, a substituted di-tolyl ether is produced.

On the other hand, when diploicin is heated with hydrobromic acid in acetic acid solution, the lactone bridge is opened and the product is simultaneously decarboxylated and demethylated, giving rise to the substituted di-tolyl ether XI.



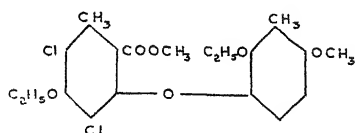
The methylated di-tolyl ether X is resistant to the action of alcoholic potash, but the demethylated product XI is readily attacked, and fission of the oxygen bridge takes place. On the assumption that the process is hydrolytic, ring B in XI could give rise to the following products:—



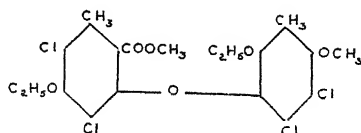
while ring A should give 4·6-dichlor-ornanol.

The structure XV, as a possible product, was excluded in the following manner by Nolan (see article by T. J. Nolan on Lichen Substances, Thorpe's Dictionary of Chemistry, 1946, p. 299).

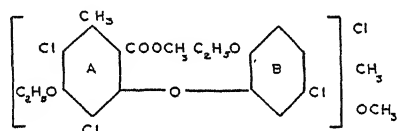
From gangalcoidin was prepared the substance



which on chlorination gave



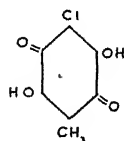
melting point 76° – 77° C. which was not identical with the compound



melting point 94° – 95° C., prepared from diploicin by treatment with methyl alcoholic potash, followed by treatment with diazo-ethane.

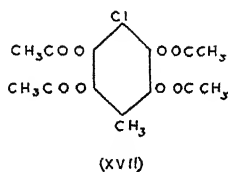
It follows therefore, that the possible products of hydrolysis arising from ring B are XII, XIII or XIV, which, in their turn, might by a process of oxidation give rise to a toluquinone containing chlorine and hydroxyl groups

When the di-tolyl ether XI was subjected to the action of methyl alcoholic potash, in the presence of oxygen, the only pure product isolated was a mono-chlor di-hydroxy toluquinone $C_7H_5O_4Cl$. On the assumption that this toluquinone originated from 2·4-dichlor trihydroxy toluene XII it could have the formula



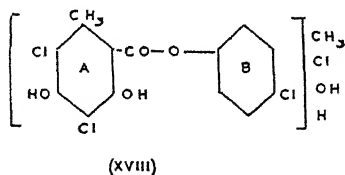
(XVI)

and, for reasons stated below, it seems certain that the substance is correctly represented by this structure. This toluquinone crystallises in very characteristic garnet-coloured hexagonal plates which give a violet coloration with a dilute solution of sodium carbonate. On reduction with sulphur dioxide and subsequent acetylation it gave the tetracetate of 4-chlor-2·3·5·6-tetrahydroxy toluene XVII, which melts at 219° C.



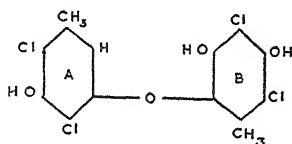
It was essential, therefore, to determine whether the toluquinone obtained from the di-tolyl ether XI, originated from 2·4-dichlor trihydroxy toluene XII, 2·6-dichlor trihydroxy toluene XIII, or 2·3-dichlor trihydroxy toluene XIV. The latter three compounds were prepared by us, and their synthesis is described in the experimental part. On examining their behaviour with methyl alcoholic potash, it was found impossible to isolate a toluquinone from the products of the reaction with 2·6-dichlor trihydroxy toluene or with 2·3-dichlor trihydroxy toluene. On the other hand, when 2·4-dichlor trihydroxy toluene was treated with methyl alcoholic potash, we were able to isolate a mono-chlor dihydroxy toluquinone, which crystallised in garnet coloured hexagonal plates and gave a violet coloration with dilute sodium carbonate. On reduction with sulphur dioxide and subsequent acetylation it gave the tetracetate of 4-chlor-2·3·5·6-tetrahydroxy toluene M.P. 219°C.

Further confirmation of the structure of diploicin was obtained in the following manner. When diploicin, in benzene solution, was heated with anhydrous aluminium chloride, a product was obtained which appeared to have the structure XVIII.

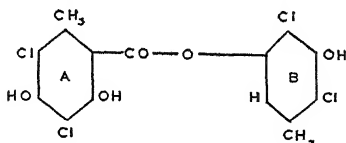


This substance formed a trimethyl ether and a triacetate, and on treatment with methyl alcoholic potash gave two products. One of these was the methyl ester of 4·6-dichlor orsellinic acid VI, and originated from ring A. The second product, derived from ring B, crystallised in garnet-coloured hexagons, identical with XVI, as on reduction and acetylation it gave the tetracetate of a mono-chlor tetrahydroxy toluene, M.P. 219°C., the mixed melting point of which with XVII was unchanged. These facts appear definitely to exclude the possibility of the toluquinone having its origin in ring A, since 4·6-dichlor orsellinic ester remains practically unaltered when treated with methyl alcoholic potash under similar conditions (see Nolan and Murphy, Sc. Proc. R.D.S. 1940, **33**, p. 317).

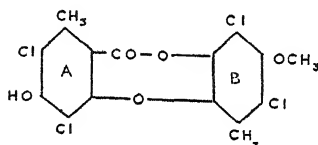
It is clear, therefore, that the same toluquinone was obtained by the action of methyl alcoholic potash on the di-tolyl ether XI, on the compound XVIII, and on 2·4-dichlor trihydroxy toluene XII. The groupings present in the latter substance must therefore be present in the di-tolyl ether XI, which may therefore be formulated



2·4·3'·5'-tetrachlor-3·2'·4'-trihydroxy-5·6'-dimethyl diphenyl ether and the structure of XVIII may be represented by

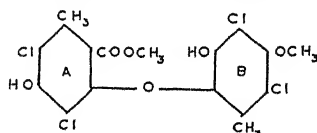


(2·4-dichlor-3-hydroxy-5-methyl) phenyl ester of 4·6-dichlor-orsellinic acid
Consequently the formula for diploicin must be



EXPERIMENTAL PART.

Action of Chlorine followed by reduction and hydrolysis on the Compound V.



M

To a solution of the above compound (0·5 g) in chloroform (60 c.c.) was added 13·3 c.c. of a solution of chlorine in carbon tetrachloride containing 0·04 g. chlorine per c.c. After standing for 24 hours in a stoppered flask, the excess chlorine was removed by blowing dry air through the solution, and the solvents removed by evaporation. The residue was a brownish gum. The latter was reduced in acetic acid solution (25 c.c.) with stannous chloride (1 g.) in twenty per cent. hydrochloric acid (30 c.c.). The latter solution was added in portions at a temperature of about 40°C. A precipitate was formed immediately. After standing overnight, water (50 c.c.) was added and the supernatant liquid decanted from the solid, which was then washed with a little five per cent. hydrochloric acid, followed by water, and air dried. The solid was refluxed gently with ten per cent. methyl alcoholic potash (25 c.c.) in an atmosphere of hydrogen for one hour. The solution was then diluted with water, acidified, and extracted with ether. The ether solution was successively extracted with three per cent. solutions of (a) sodium bicarbonate and (b) sodium carbonate.

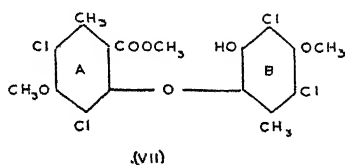
(a) The sodium bicarbonate extract was acidified and the precipitated material subjected to steam distillation, giving a white crystalline material in the distillate. On crystallisation of this material from aqueous methyl alcohol, colourless crystals were obtained M.P. 115°C ., identical with the 4·6-dichlor orsellinic acid methyl ester (VI) prepared by Nolan and Murphy (*loc. cit*)

Found — $\text{C}_{40}\cdot 2$, $\text{H}_3\cdot 8$, $\text{Cl } 26\cdot 0$.

$\text{C}_9\text{H}_8\text{O}_4\text{Cl}_2$, H_2O requires — $\text{C}_{40}\cdot 14$, $\text{H}_3\cdot 71$, $\text{Cl } 26\cdot 4$.

(b) Acidification of the sodium carbonate extract mentioned above, gave the original substance V, unaltered.

Action of Chlorine followed by reduction and hydrolysis on the Compound VII.



The above compound (0·55 g) was dissolved in chloroform (30 c c) and to it was added a solution of chlorine in carbon tetrachloride (6·5 c.c.) containing 0·085 g. of chlorine per c.c. The mixture was allowed to stand five days in a stoppered flask, excess chlorine was removed in a stream of dry air, and the solvents removed by evaporation. The residual oil was dissolved in acetic acid (40 c.c.) and reduced with stannous chloride (1·2 g.) dissolved in twenty per cent. hydrochloric acid (40 c.c.). After standing overnight, the mixture was diluted with water and extracted with chloroform. After washing with water, the chloroform solution was evaporated, leaving an oil. The latter was refluxed with ten per cent. methyl alcoholic potash (30 c.c.) for one hour, in a hydrogen atmosphere. The mixture thus obtained was cooled, diluted with water, acidified with dilute sulphuric acid, and extracted with ether. The ether solution was successively extracted with (a), three per cent. sodium bicarbonate and (b), one per cent. sodium hydroxide.

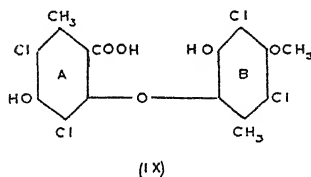
(a) Acidification of the sodium bicarbonate solution gave a brown gum which could not be obtained in a crystalline condition.

(b) The sodium hydroxide extract was acidified with dilute sulphuric acid and extracted with ether. Evaporation of the ether gave an oil which was repeatedly treated with hot petroleum ether (B.P. 60° – 80°C .). On cooling, the solvent deposited crystals, which were recrystallised twice from aqueous methyl alcohol, giving colourless needles, M.P. 78° – 79°C .. The latter gave a violet coloration with ferric chloride, and the substance was identical with the synthetic 4·6-dichlor orsellinic acid methyl ester 5-monomethyl ether (VIII) described by Nolan and Murphy (*loc. cit*).

Found — $\text{C}_{45}\cdot 1$, $\text{H}_3\cdot 9$, OCH_3 23·9.

$\text{C}_9\text{H}_4\text{O}_2\text{Cl}_2(\text{OCH}_3)_2$ requires :— $\text{C}_{45}\cdot 3$, $\text{H}_3\cdot 8$, OCH_3 23·4.

2·4·3'·5'-tetrachlor—3·2'-dihydroxy—6-carboxy—4'-methoxy—5,6'-dimethyl
diphenyl ether IX.

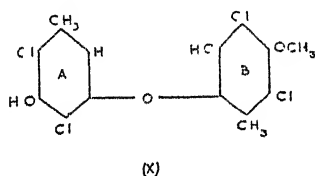


Diploicin was hydrolysed by heating to gentle boiling with two per cent. aqueous sodium hydroxide. The liquor at first was pale yellow, afterwards slightly brownish. The heating was continued until practically all the solid had gone into solution (ca. fifteen minutes), then cooled, filtered, and acidified with dilute hydrochloric acid. The precipitate obtained was filtered, washed with water, and air dried. The product was crystallised several times from boiling benzene, and then dried in the air oven at 120°C. for several hours. The crystals thus obtained occurred both as colourless rhombohedra and as needles. M.P. 216°–218°C (decomp.).

Found:— C43·93, H3·02.

C₁₆H₁₂O₆Cl₄ requires — C43·44, H2·71

2·4·3'·5'-tetrachlor—3·2'-dihydroxy—4'-methoxy—5,6'-dimethyl diphenyl ether X



This compound was obtained by the elimination of carbon dioxide from the carboxylic acid IX above. Decarboxylation takes place very readily, and can be achieved in either of two ways.

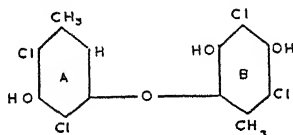
Method I. The hydrolysis of diploicin described above was continued for a period of about one hour. When the solution thus obtained was saturated with carbon dioxide, most of the material was precipitated as a white solid, which did not contain a carboxyl group and had the di-tolyl ether structure X. Crystallisation from benzene gave colourless needles M.P. 186°C.

Method II. The carboxylic acid IX was heated above its melting point, when decomposition occurred with evolution of carbon dioxide. The resultant brown product was crystallised from dilute alcohol (norite) and afterwards from benzene, and was obtained as colourless needles M.P. 186°C., identical with the compound obtained by method I. The substance gives no colour with ferric chloride.

Found :— C 45·45, H 3·1, Cl 35·4.

C₁₅H₁₂O₄Cl₄ requires :—C 45·23, H 3·02, Cl 35·68.

2·4·3'·5'-tetrachlor—3·2'·4'-trihydroxy—5·6'-dimethyl diphenyl ether XI.



(XI)

This compound was produced by either of the following procedures.

Method I. A mixture of the methoxy derivative X (9 g.), acetic acid (180 c.c.), constant boiling hydrobromic acid (150 c.c.) and red phosphorus (18 g.), was boiled under reflux for eight hours and was then cooled and filtered. The filtrate, on diluting largely with water, gave a small amount of cream precipitate. The residue of red phosphorus was extracted several times with boiling alcohol, filtered, and the alcoholic filtrate diluted with water, giving a considerable amount of white precipitate. The latter was crystallised from dilute alcohol, and furnished colourless, hexagonal crystals, M.P. 235°C.

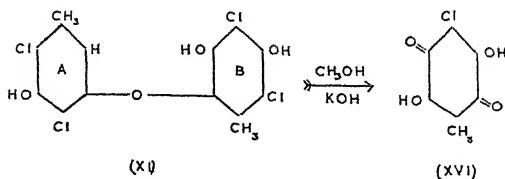
Method II. Diploicin (1 g.) in acetic acid (20 c.c.) was heated with red phosphorus (2 g.) and constant boiling hydrobromic acid (16 c.c.) for eight hours, under reflux. After cooling the liquor was filtered, and the phosphorus washed with acetic acid. The whole of the filtrate was diluted with water and saturated with sodium bisulphite in order to decolorise it. A cream coloured precipitate was obtained. Further quantities of material were obtained by extraction of the phosphorus with hot alcohol. Crystallisation of the crude substance from hot dilute alcohol and boiling benzene gave colourless hexagons, M.P. 235°C. The mixed melting point of this substance with that obtained by method I was unchanged. Yield about 60 per cent.

Found :— C 44·53, H 2·96, Cl 36·35.

C₁₄H₁₀O₄Cl₄ requires :— C 43·7, H 2·6, Cl 37·0.

Action of methyl alcoholic potash on 2·4·3'·5'-tetrachlor—3·2'·4'-trihydroxy—5·6'-dimethyl diphenyl ether XI.

Production of the toluquinone XVI.



(XV)

(XVI)

The compound XI (1.3 g.) was treated with five per cent. methyl alcoholic potash (30 c.c.), and heated under reflux at 60°C. for about three hours. Throughout this period, a slow stream of oxygen was bubbled through the solution. On standing overnight, the dark brown potassium salt of the quinone separated. This was filtered off, washed with a little methyl alcohol, dissolved in water, and acidified with dilute hydrochloric acid. The quinone was extracted with ether, the ether solution dried over anhydrous sodium sulphate, filtered, and evaporated to dryness, leaving a red crystalline residue. The latter was dissolved in the minimum volume of warm ethyl acetate, a large excess of chloroform added and the mixture allowed to stand. After a short time, garnet coloured hexagonal crystals of the quinone separated. The quinone sinters about 230°C., becomes black and melts about 264°C. (decomp.). The substance gives a violet colour with one per cent. aqueous sodium carbonate.

Found :— C 44.57, H 2.72, Cl 19.1.

$C_7H_5O_4Cl$ requires :— C 44.55, H 2.65, Cl 18.8.

Tetracetate of 4-chlor—2.3.5.6-tetrahydroxy toluene XVII.

The toluquinone XVI was suspended in water, saturated with sulphur dioxide and the mixture allowed to stand for several days, the solution then having a very pale straw colour. Extraction of this solution with ether gave crude 4-chlor—2.3.5.6-tetrahydroxy toluene M.P. ca. 183°C., which tended to darken on exposure to the air. The air dried tetrahydroxy compound was dissolved in acetic anhydride to which a drop of concentrated sulphuric acid was added. After standing one hour, the mixture was diluted with water and extracted with ether. The ether solution was washed with water and dried over anhydrous sodium sulphate. The residue obtained on evaporation of the ether was crystallised from a mixture of benzene and ligroin and gave colourless prisms M.P. 219°C.

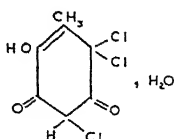
Found :— C 50.15, H 4.36, Cl 10.38.

$C_{15}H_{15}O_8Cl$ requires :— C 50.21, H 4.18, Cl 9.9.

2.4-dichlor—3.5.6-trihydroxy toluene XII.

To obtain this substance use was made of an observation of Heinrich, Meyer and Droschky (Ber. 37, 1904, p. 1427) that the action of hypochlorous acid on β -amino-orscin (which is 2-amino-orscin) gives rise to a product of formula $C_7H_7Cl_2O_4$. Heinrich did not attribute a structure to this substance, but the work of Zincke and his co-workers

(Zincke and Schurmann, Ann 1918, 417, pp. 236-256) on the keto-chlorides suggested to us that it was a keto-chloride $C_7H_5Cl_3O_3$, H_2O to which the following formula, or one of similar type might be attributed.—



Heinrich's product was obtained by a modification of his method and on reduction with stannous chloride, it gave rise to a dichlor-trihydroxy toluene, for which the only possible formula appears to be XII above.

(a) *Chlorination of 2-amino-orscin* 2-amino-orscin (4.7 g.) was added to a cooled mixture of acetic acid (18.8 c.c.) and concentrated hydrochloric acid (9.2 c.c.) and chlorine was passed through the mixture to saturation. The liquid was allowed to stand overnight and then poured into a dish and placed in a draught to remove excess chlorine. An equal volume of water was added and the solution cooled, when the yellow crystalline compound which was precipitated was filtered, washed with a little water and dried over sodium hydroxide in vacuo. (yield 2.2 g.). It was crystallised from benzene giving colourless crystals (0.6 g) M.P. 92° – 95° C. On addition of a little water to the benzene mother liquors, colourless needles of a hydrate crystallised out (0.65 g.). Softens 92° C., M.P. 96° – 98° C.

Analysis of this hydrate gave the following results —

Found :— C 31.0, H 2.75, Cl 38.75.

$C_7H_5Cl_3O_3$, $1\frac{1}{2} H_2O$ requires :— C 31.05, H 2.88, Cl 39.4.

(b) *Reduction of the keto-chloride.* The keto-chloride (0.8 g.) was dissolved in acetic acid (4 c.c.). To the solution stannous chloride (2.8 g.) in concentrated hydrochloric acid (10 c.c.) was added slowly, with cooling. A white crystalline mass separated. The mixture was allowed to stand overnight, in the dark, in a stoppered flask when the crystals were filtered, washed with 1 : 1 hydrochloric acid-water and air dried. Recrystallisation from benzene gave pale pinkish-brown needles M.P. 168° – 172° C. The substance gives a port-wine colour with ferric chloride and a cerise coloration with one per cent. sodium carbonate.

Found.— C 40.3, H 2.92, Cl 33.5.

$C_7H_6O_3Cl_2$ requires :— C 40.2, H 2.87, Cl 34.0.

Action of methyl alcoholic potash on 2.4-dichlor-3.5.6-trihydroxy toluene XII.

Production of the toluquinone XVI.

2.4-dichlor-3.5.6-trihydroxy toluene (3 g.) was dissolved in five per cent. methyl alcoholic potash (60 c.c.). A transient green colour was produced which

rapidly changed to deep purple. The solution was heated under reflux at 40°–50°C. for two hours, while a slow stream of oxygen was bubbled through. On standing overnight, the dark brown precipitate which had separated was filtered, washed with a little methyl alcohol, dissolved in water and acidified with dilute hydrochloric acid. The liquor was extracted with ether, the ether dried over anhydrous sodium sulphate and evaporated, leaving a reddish residue. The residue on crystallisation from ethyl acetate-chloroform gave garnet coloured hexagonal crystals sintering about 230°C., which gave a violet coloration with one per cent. sodium carbonate.

Found — C 44.24, H 3.2, Cl 18.7

$C_7H_5O_4Cl$ requires — C 44.55, H 2.65, Cl 18.8.

The identity of this substance with the toluquinone XVI, obtained by the action of methyl alcoholic potash on the di-tolyl ether XI, was shown in the following way. Reduction of the substance followed by acetylation gave the tetracetate of 4-chlor-2.3.5.6-tetrahydroxy toluene M.P. 219°C.

Found :— Cl 9.36, $C_{15}H_{15}O_8Cl$ requires — Cl 9.9.

The mixed melting point of this tetracetate with XVII was unaltered.

2.6-dichlor—3.4.5-trihydroxy toluene XIII.

In the production of this compound, considerable difficulty was experienced in preparing the necessary starting substance, 3.4.5-trihydroxy toluene. Various methods were tried of which the following two proved the most satisfactory.

Method I. 3.4.5-trihydroxy toluene was prepared from atranol (2.6-dihydroxy-4-methyl benzaldehyde) by the Dakin reaction in accordance with the procedure described by St. Pfau (Helv. Chim. Acta, 9, p. 667) and was obtained as brown needles M.P. 125°–130°C. Yield 25 per cent.

Method II. The procedure described by Asahina (Ber. 69, 1936, p. 2328) was more convenient and gave somewhat better yields than method I. Galloyl chloride trimethyl ether was reduced by Rosenmund's method to gallic aldehyde trimethyl ether. The latter was reduced by hydrazine hydrate to 3.4.5-trimethoxy toluene which was then demethylated with acetic acid-hydrobromic acid and furnished brown needles of 3.4.5-trihydroxy toluene M.P. 119°C.

Chlorination of 3.4.5-trihydroxy toluene. 3.4.5-trihydroxy toluene (1 g.) prepared by either of the above methods, was dissolved in dry ether (15 c.c.) and sulphuryl chloride (2.1 g. in 5 c.c. ether) was added. A maroon colour developed and slight evolution of heat took place. After standing one hour, the solution was shaken with a little water. The ether solution was dried over anhydrous sodium sulphate, filtered, and evaporated in vacuo, leaving a pale brown residue. Crystallisation from benzene gave 0.6 g. pinkish white crystals, which did not melt sharply, but over a range of 170°–180°C.

Found — Cl 33.7, $C_7H_5O_3Cl_2$ requires :— Cl 33.9.

With one per cent. sodium carbonate 2·6-dichlor-3·4·5-trihydroxy toluene gave a cerise coloration, turning brown on standing. With aqueous ferric chloride it gave a cobalt blue colour, fading to pale blue on standing.

Action of methyl alcoholic potash on 2·6-dichlor-3·4·5-trihydroxy toluene XIII.

2·6-dichlor-3·4·5-trihydroxy toluene was treated with five per cent. methyl alcoholic potash. The solution, initially pink, changed to blue and finally brown. The mixture was heated under the same conditions as described for the treatment of 2·4-dichlor-3·5·6-trihydroxy toluene above and the separated solid worked up in a similar manner. The only product obtained was a brown oil, which could not be induced to crystallise. No material of quinone type could be isolated from the reaction mixture.

2·3-dichlor-4·5·6-trihydroxy toluene XIV.

2·3·4-trihydroxy toluene was obtained by Clemmensen reduction of pyrogallol o-aldehyde (Dimroth and Zoeppritz, Ber. 35, 1902, p. 996) according to the method of Majima and Okazaki (Ber. 49, 1916, p. 1492) and crystallised from benzene in off white needles M.P. 140°C. Chlorination of this trihydroxy toluene was effected with sulphuryl chloride as described for the production of XIII. Chlorination did not appear to proceed so smoothly as in the latter case, but material was obtained which crystallised from benzene in faintly pink oval plates, M.P. 120°C. (decomp.). With one per cent. sodium carbonate it gave a yellow colour, turning brown rapidly. Alcoholic ferric chloride gave a pale green coloration, turning olive green rapidly.

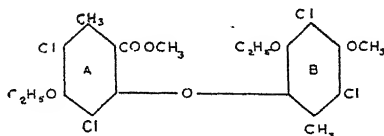
Found :— Cl 36·7, $C_7H_6O_3Cl_2$ requires :—Cl 33·9.

Although somewhat impure, the substance appeared to be the required dichloro derivative and was employed, without further purification in the following experiment.

Action of methyl alcoholic potash on 2·3-dichlor-4·5·6-trihydroxy toluene XIV.

2·3-dichlor-4·5·6-trihydroxy toluene was warmed with five per cent. methyl alcoholic potash under conditions similar to those described above for XII and XIII. Only brown oily material, which could not be induced to crystallise, was isolated from the reaction product. There was no indication of the formation of a quinone.

2·4·3'·5'-tetrachlor—3·2'-diethoxy—4'-methoxy—6-carbomethoxy—5·6'-dimethyl
diphenyl ether.



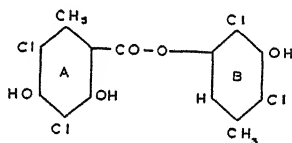
Treatment of the substance V with diazo-ethane and crystallisation of the product from methyl alcohol gave the above compound as colourless crystals M.P. 94°–95°C.

Found — C 48·63, H 4·1, Cl 27·9

$C_{15}H_6O_2Cl_4(OCH_3)_2(OC_2H_5)_2$ requires — C 49·2, H 4·3, Cl 27·7.

This compound cannot be identical with the tetrachloro derivative obtained from gangaleoidin and mentioned in the introductory part, as the latter melts at 76°–77°C.

(2·4-dichlor—3-hydroxy—5-methyl) phenylester of 4 6-dichlor—orsellinic acid XVIII.



Diploicin (5 grms) was dissolved in the minimum volume of boiling dry benzene anhydrous aluminium chloride (7 grms) was added and the mixture refluxed on the steam bath for six hours. After standing overnight, the solvent was decanted and the residue decomposed with ice and dilute hydrochloric acid. It was then extracted with ether and the ether solution extracted several times with dilute sodium bicarbonate. Acidification of the sodium bicarbonate solution gave a white precipitate, which was washed with water, air dried and crystallised from benzene, in which it was sparingly soluble. The pure substance crystallises in pale yellow prisms M.P. 206°C. Yield 2·8 grms. The compound gives a faint reddish brown colour with ferric chloride and a yellow colour with bleaching powder solution. It dissolves in dilute sodium carbonate to a faintly yellow solution which does not give a violet colour on standing.

Found :— C 42·09, H 2·52, Cl 32·6.

$C_{15}H_{10}O_6Cl_4$ requires — C 42·05, H 2·33, Cl 33·1.

Diploicin was recovered unchanged from the material insoluble in dilute sodium bicarbonate.

Acetylation of the compound with acetic anhydride and a trace of sulphuric acid gave a triacetate which crystallised from benzene and ligroin in colourless prisms M.P. 175°–176°C.

Found — C 45.99, H 2.89, Cl 25.8.

$C_{13}H_7O_6Cl_4(CH_3CO)_3$ requires — C 45.49, H 2.88, Cl 25.7.

Methylation of the compound with diazo-methane gave a trimethyl ether which crystallised from methyl alcohol in colourless prisms M.P. $184^{\circ}C$.

Found :— C 47.36, H 2.92, Cl 31.3.

$C_{13}H_7O_2Cl_4(OCH_3)_3$ requires .— C 47.57, H 3.5, Cl 31.2.

Action of methyl alcoholic potash on the compound XVIII.

Compound XVIII (1.3 grms) in five per cent. methyl alcoholic potash (40 c c) was heated at 60° – $70^{\circ}C$. for four hours while a slow stream of oxygen was bubbled through the solution. After standing overnight, the mother liquor was filtered from precipitated dark brown solid. The latter was dissolved in a little water, acidified with dilute hydrochloric acid and extracted with ether. Evaporation of the ether gave a reddish brown residue which crystallised from ethyl acetate-chloroform in garnet coloured hexagons (0.1 grm.), which sintered at $230^{\circ}C$. The identity of this compound with the toluquinone obtained from the ditolyl ether XI and from 2.4-dichlor-trihydroxy toluene XII was shown in the following manner. The substance was reduced with aqueous sulphur dioxide and the resultant product acetylated. The tetracetate thus obtained melted at $219^{\circ}C$. and was identical (mixed melt) with the compound XVII previously described.

The mother liquor, above, from the dark brown solid was saturated with carbon dioxide, diluted with water and extracted with ether. On evaporation of the ether and crystallisation of the residue from aqueous alcohol, colourless needles were obtained M.P. 120° – $121^{\circ}C$., giving a violet coloration with ferric chloride. Analysis showed this substance to be the methyl ester of 4.6-dichlor-orsellinic acid VI, containing one molecule of water of crystallisation. A mixed melting point with the methyl ester of 4.6-dichlor-orsellinic acid (M.P. 115° – $117^{\circ}C$.) synthesised by Nolan and Murphy (*loc. cit.*) melted at 115° – $117^{\circ}C$.

Found — C 39.48, H 4.01, Cl 26.3

$C_9H_8O_4Cl_2 \cdot H_2O$ requires .— C 40.14, H 3.71, Cl 26.4

That this substance was actually the methyl ester of 4.6-dichlor-orsellinic acid VI was shown in the following way. The compound was acetylated and gave a diacetate which crystallised from aqueous alcohol in colourless prisms M.P. $93^{\circ}C$. and gave no colour with ferric chloride.

Found :— C 46.66, H 3.44, Cl 21.6.

$C_9H_6O_4Cl_2(CH_3CO)_2$ requires :— C 46.56, H 3.58, Cl 21.2.

The mixed melting point of this diacetate with the diacetate of 4.6-dichlor-orsellinic acid methyl ester, prepared in the manner described below, was unaltered.

A specimen of o-orsellinic acid methyl ester from lecanoric acid was chlorinated with sulphuryl chloride to give crude 4.6-dichlor-orsellinic acid methyl ester (M.P. ca $115^{\circ}C$.). The latter was acetylated and gave on crystallisation from aqueous alcohol a diacetate M.P. $93^{\circ}C$.

No. 29.

SALMON AND SEA TROUT OF THE RIVER INNY.

By

ARTHUR E. J. WENT.

(PLATE 18)

(Read NOVEMBER 25, 1947. Published Separately, SEPTEMBER 6, 1948).

THE River Inny in County Kerry is a small spate river, about 16 miles long, with a catchment area of only 47 square miles (see Fig 1). Its catchment area is entirely on the Old Red Sandstone, and it drains some very high ground (over 2,000 feet). As limestone is absent from the catchment area the waters of the river are always acid, the extent of the acidity depending to some degree on the height of the water. An analysis of the waters of the river when it was at an average level is given in TABLE 1 (See Appendix for all tables)

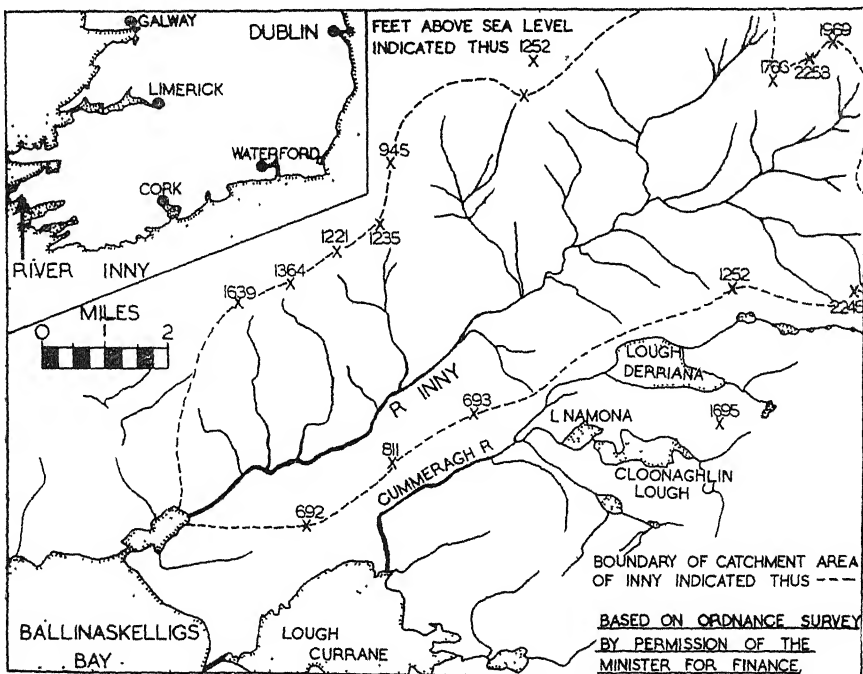


Fig. 1. Sketch map of the Inny watershed.

Apart from affording sport to the angler the Inny supported a commercial net fishery, partly in fresh water and partly in the tideway. Netting in the freshwater portion of the river has, however, been abolished under the provisions of Section 35 of the Fisheries Act, 1939, as from 1st January, 1948. The material on which this investigation was based was collected in the years 1941, 1942, 1943 and 1944 by the owner of one of the nets fishing in the river, and consisted of 747 satisfactory sets of scales and data relating to salmon, and 217 sets relating to sea trout. Unfortunately it was not found possible to weigh the fish. The distribution of the samples over the various months of the season is indicated in Table 2.

AGE AND GROWTH OF THE SALMON.

Smolt ages. The distribution of the various smolt classes has been given in Table 3. Four smolt classes were present. The proportion of the three-year smolt class was very high when compared with the results from other Irish rivers (see Went, 1947, *Sci. Proc. R. Dublin Soc.* 24 (N.S.) No. 19 p. 171). The proportion of old smolts (three and four years old) tends to decrease as the age groups are ascended. In other words there was a decrease in the average age of the smolt as the age groups were ascended.

Smolt types. When dealing with salmon of other Irish rivers (see bibliography, Went, 1947, *op. cit.*) it was mentioned that the smolts could be divided into two types, namely, (1) Type B. smolts which showed two or more growth elements on the scales corresponding to growth in fresh water in the spring prior to migration to the sea as a smolt, and (2) Type A. smolts which made no such growth. In the present material a similar division was made and the results are given in Table 4.

Age groups. The fish were divided into three groups of maiden or unspawned fish and one group of fish which had only one common character, namely the presence of one or more spawning marks on their scales. The results have been given in Table 5. The bulk of the fish were grilse (1 + winters) with the other three age groups each forming 7.0% or less of the total. The proportion of previous spawners was low (3.1%), being considerably lower than the average for Ireland in 1944 and 1945 (see Went, 1947, *op. cit.* p. 175).

May was the best month for small spring and summer fish (2 and 2 + winters) and July for grilse and previous spawners (Table 6). In April all the fish were spring fish, in May 39.1% were spring fish, in June 10.8%, and thereafter the runs consisted almost entirely of summer fish (Table 7).

Twenty-three previous spawners were examined, and they all exhibited the "short absence" habit, that is to say, the period feeding in the sea between successive entries into fresh water was less than a full year. Twenty-one of the previous spawners had spawned once previously, one twice, and one three times. The fish which had spawned three times previously, measured 35 inches in length, and was captured on 19 July, 1941. Fish of this type in Ireland are exceedingly rare. As

mentioned in a previous publication (Went, "The Value of the kelt" *Salmon and Trout Magazine* No 119, Jan. 1947. p. 45) only two previous specimens have been recorded from Ireland. A photograph of one of the scales of this fish is given in Plate 18

Divided migration and return. In Table 8 the years in which the different fish were hatched have been indicated. Five-year classes, i.e. the broods of five years, were responsible for the runs in one year, but over 96% of the fish belonged to two year-classes.

Size distribution. The distribution of the various size groups has been given in Table 9, and is illustrated in Fig. 2. As would have been expected in a grilse river the fish were concentrated in a few length groups, almost $\frac{3}{4}$ of the fish having lengths between 21.95 and 26.95 inches.

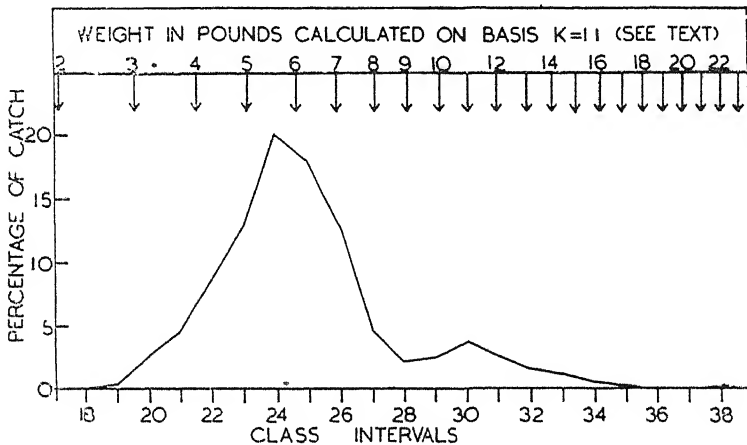


Fig. 2. Estimated size distribution of the Inny salmon

Average sizes. Unfortunately the person collecting the material was not prepared to weigh the fish, and therefore we have had to be content with the length measurements only. Details of the average lengths in the different smolt classes of the different age groups are given in Tables 10-12, no attempt having been made to give the monthly averages, except in the case of the grilse, as the numbers of other age groups were small

(a) Grilse (1 + winters). Number examined = 640.

| | | INCHES | DATE OF CAPTURE | SMOLT CLASS |
|---------|------|--------|-----------------|-------------|
| Minimum | | 18.6 | 3/7/'42 | 2 year |
| Average | | 24.5 | — | — |
| Maximum | ... | 30.3 | 19/7/'41 | 2 year |
| | | 30.3 | 15/9/'41 | 2 year |

From June onwards the average length of the grilse increased slightly as the season progressed.

(b) Small spring fish (2 winters). Number examined = 32.

| | INCHES | DATE OF CAPTURE | SMOLT CLASS |
|-------------|--------|-----------------|-------------|
| Minimum .. | 26.4 | 11/5/'43 | 2 year |
| Average . . | 32.4 | — | — |
| Maximum ... | 35.1 | 30/5/'41 | 2 year |

(c) Small summer fish (2 + winters). Number examined = 52.

| | INCHES | DATE OF CAPTURE | SMOLT CLASS |
|-------------|--------|-----------------|-------------|
| Minimum . | 27.5 | 7/6/'41 | 2 year |
| Average . . | 31.4 | — | — |
| Maximum . | 38.0 | 15/9/'41 | 2 year |

The largest fish of this age group was the largest fish of which scales were examined in this investigation and probably weighed about 22 pounds. (See Fig. 2.)

(d) Previous spawners (with SMs). Number examined = 23.

| | INCHES | DATE OF CAPTURE | SMOLT CLASS |
|-------------|--------|-----------------|-------------|
| Minimum . | 24.3 | 23/6/'44 | 1 year |
| Average . . | 29.4 | — | — |
| Maximum .. | 35.0 | 19/7/'41 | 2 year |

The smallest previous spawner had spawned only once previously, whereas the largest fish of this type had spawned three times previously (see page 336 and Plate 18).

In order to give some idea of the weights of the fish involved a rough scale has been added to Fig. 2. This scale has been drawn up on the basis of a value of 1.1 for the *Condition Coefficient* on Menzie's scale, the *Condition Coefficient* (K) being calculated from the formula $K = 100,000W/L^3 \times 36$. (W = weight in lbs. and L = length in inches).

Erosion. After a sojourn in fresh water the material of a scale of a salmon is partly absorbed, particularly at its margin. In the present material a proportion of the fish had eroded scales (see Table 13). In each age group the proportion of fish having eroded scales and the average degree of erosion increased as the season progressed. Erosion in the grilse (1 + winters) was not as advanced as in the other age groups.

Calculated lengths. The lengths of each maiden or unspawned fish at the end of every year of life were calculated in the usual way from the measurements of the scales, i.e. on the assumption that the growth of the scale is strictly in proportion to the growth of the fish.

River life. The relationships of the parr lengths are dealt with in this section. In Table 14 the growth in freshwater has been indicated for each smolt class (see also Fig. 3.). The fastest growing parr migrated first. At the end of the first year the one-year smolts were markedly longer than those of the other three smolt classes. Similar conditions have already been described for other Irish rivers (see bibliography Went, 1947, *op. cit.*). The length of the smolt was also calculated, and it

was shown that the mean smolt length increased as the smolt classes were ascended. (Table 14) The distribution curves for the two year smolt class have been added to Fig. 3.

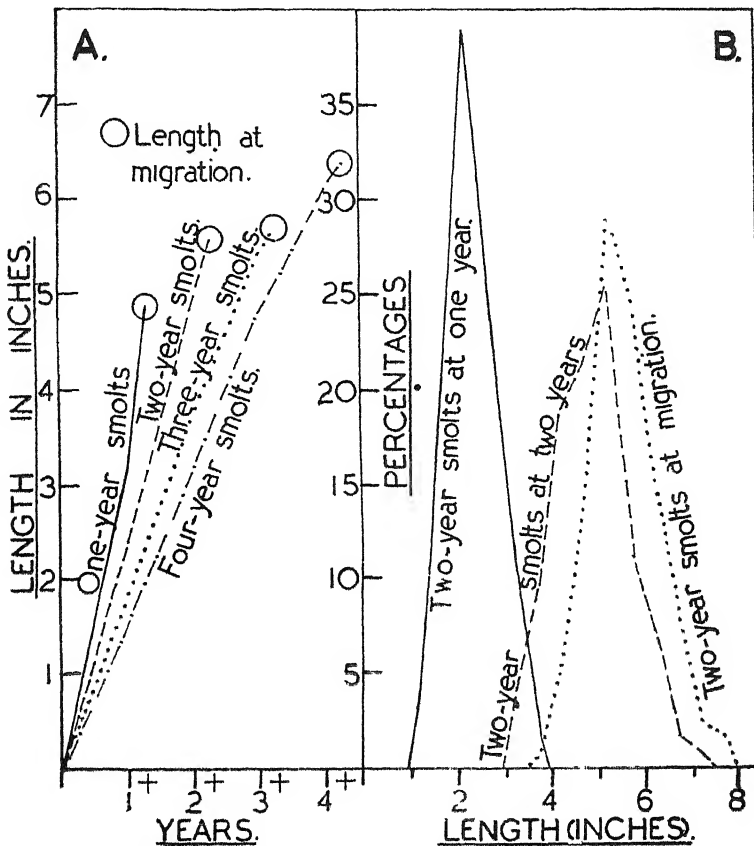


Fig 3 A Growth of Inny salmon in fresh water
B Distribution curves for the two-year smolts at one-year, at two years and at migrations as percentages of the total.

As was mentioned on page 336 the smolts could be divided into two types namely Type A and Type B smolts. The growth in each type in each smolt class is given in Table 15. In general the Type A smolts were longer at the end of any one year than the Type B smolts of the same year class.

In Table 12 the growth in fresh water of the different age groups has been given, and there appears to be no marked differentiation between the different age groups.

Sea life. The relationships of the lengths at the end of every year of sea life are dealt with in this section. In Tables 11 and 12 the lengths of the different age groups at the end of every year of sea life have been given. Taken as a whole the grilse had slightly superior lengths at the end of the first sea winter, but the differences are small and may not be significant. At the end of the second sea winter

the small spring fish (2 winters) were longer than the small summer fish, (2 + winters), so much so in fact that although on the average, the small summer fish put on 2.4 inches between the end of the winter and capture, they were still smaller than the small spring fish.

NOTES ON THE SEA TROUT.

As mentioned earlier a small amount of material relating to sea trout was collected from the River Inny during the years 1941 to 1944. This material, consisting of sets of scales with relevant data from 217 fish, cannot be regarded as completely adequate, quite apart from the question of its paucity. The fish, from which the material was obtained were all captured by means of a drift net with a mesh of 7 inches in the round ($1\frac{3}{4}$ inches knot to knot). Such a net is clearly selective, as many of the smaller sea trout, particularly whiting or finnock (fish in their first post-migration year) escape capture. Nevertheless bearing this in mind the results appear to be worth recording.

Smolt ages. In Table 16 the distribution of the different smolt ages in the various age groups has been given for the maiden or unspawned fish and the previous spawners together. The two-year smolts were the most important forming over three-quarters of the total, the second most important class being the three-year smolts (about one-seventh of the total). The proportion of one-year smolts was very high (cf. G.H. Nall, 1930. *The life of the sea trout* p. 311) but it must be remembered that this river is much further south than the majority of rivers investigated heretofore.

Smolt types. When dealing with the salmon (page 336) it was mentioned that the fish could be divided into two types on the basis of the growth made in fresh water in the spring before migration to the sea as a smolt. A similar division can be made in the sea trout also (Table 17). The bulk of the fish belonged to the type B smolts and the proportion of type A smolts increased as the smolt classes were ascended (Table 17).

Age groups. In Table 18 the fish have been classified on the basis of the period of sea feeding. It will be noted that fish in their second post migration year were in the majority. The relative absence of whiting or finnock (fish in their first post-migration year) to some extent, may have been due, as explained previously, to the selective capture of the fish. This is confirmed by consideration of the previous spawners from which it was estimated that about 20% had originally spawned in their first post-migration year (Table 18). Fish in their third or fourth post-migration year were clearly unimportant.

The monthly distribution into the various age groups of the fish investigated has been given in Table 19. In general the proportion of previous spawners tends to decrease as the season progresses. Although there was, in the material at the writer's disposal, an increase in the proportion of previous spawners in July, the

large incoming populations of small maiden fish, not sampled, tend to provide runs of fish almost completely maiden in character, as we know from our observations on this river.

In the material at the writer's disposal 70.5% of the fish were maiden fish, and the remainder were previous spawners. Of the previous spawners, 64 in number, 38 (59%) had one spawning mark, 20 (31%) had two such marks and 6 (10%) had three marks. There is a suggestion (Table 16) that the older smolts tend to return to the fresh water earlier than the younger smolt groups.

Dates of the incoming populations. In Ireland it is generally assumed that there are no worth-while runs of sea-trout in most of our rivers until the end of June. Whilst this may be so in some rivers, it is obvious that in the Inny there are clean-sea-trout entering the river from the beginning of April onwards. In fact the peak of the runs of large fish would appear to be in May (Table 19).

Divided migration and return. The years in which the fish were hatched have been indicated in Table 20. In the material at the writer's disposal the fish were derived from five spawning years. Owing to the absence of the younger smolt classes in the whiting (fish in their first post-migration year) the progeny of one other spawning may have been eliminated.

Average sizes. The average sizes in the various categories of fish have been given in Table 21. The symbols used in the first column of this Table require some explanation. The first figure indicates the period of feeding in fresh water before the smolt migration, the figure or symbol after the full stop indicates the period of sea feeding (+ = fish in their first post-migration year, 1+ in their second post-migration year, etc.) and the symbols SM+ which follow indicate the number of times a fish has spawned and returned to the fresh water after a period of sea feeding. After spawning the growth rate, as might have been expected, tended to fall off very much. This is partly due to the necessity of recovering material lost at spawning, and partly due to the fact that between spawnings the period of feeding in any one year is limited to two or three months. For example, as will be seen later, between the end of the first sea winter and the date of capture, the 2.1+ category increases in length from 12.6 inches to 16.4 inches (3.8 inches) whereas between the first spawning and the second return to fresh water as a clean sea trout the increment was only 1.2 inches (from 16.4 to 17.6 inches).

In the whiting (fish in their first post-migration year) the smolt age would appear to have a considerable influence on the size of the fish. For that reason only the whiting derived from old smolts appear to be taken by the draft net used in the River Inny. In those fish which have spent longer feeding in the sea before returning to fresh water to spawn, the smolt age has a much smaller effect on the mean length of the fish (cf. 2.1+ and 3.1+ fish in which the mean lengths are 16.4 and 16.5 inches respectively). Taking all the smolt classes together the mean lengths are as follows.

| Age group | No. | Mean length in inches |
|-----------|-----|--------------------------|
| + | 3 | 13.4 |
| 1+ | 142 | 16.3 |
| 2+ | 6 | 18.1 |
| 3+ | 1 | 17.5 |

Oldest and longest fish. Nine fish in their seventh year were the oldest fish examined in this material. One of these, a fish whose life history may be indicated $3.1 + .2SM +$, had a length of 23.5 inches, which was the highest length recorded from the sea trout examined. On the basis of a condition coefficient of 1 on the Scottish standard (cf. Nall, 1930, *op. cit.* p. 315) the fish would have weighed just under 6 lb. (5.97 lb.).

Calculated lengths. The length at the end of every year of life was calculated in the normal way, i.e. on the assumption that the growth of the scale was strictly in proportion to the growth of the fish, for every maiden fish of which the scales were suitable for measurements.

River Life. The mean length in inches in the different smolt classes at the end of each year of freshwater life have been given in Table 23. As in the case of the salmon the fastest growing parr migrated first. (see also Fig. 4). The mean smolt length increased as the smolt classes were ascended (Fig. 4). The mean smolt length at 7.5 inches is considerably lower than that of the Waterville River which has been estimated to be 9.9 inches (see Went, 1944 *Sci. Proc. R. Dublin Soc.* 23 (N.S.) No. 20 page 202). Type A smolts in general grow somewhat faster than type B smolts of the same smolt class.

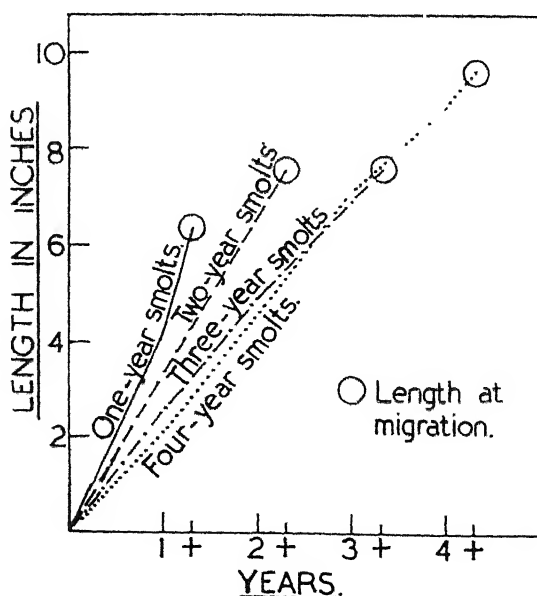


Fig. 4. Growth in fresh water of Inny sea trout.

Sea Life. Mean lengths at the end of each year in the sea in the different age groups have been given in Tables 23 and 24. As only the fish in their second post-migration year were in sufficient numbers, no conclusions can be drawn from the different mean lengths. The results are included, however, for comparison with the results from other rivers

COMPARISON OF THE GROWTH OF SALMON AND SEA TROUT IN THE RIVER INNY.

In Tables 12 and 23 growth in the various categories of salmon and sea trout have been indicated. A complete comparison of the growth in the two species is not possible, but comparable groups can be compared, i.e. 2.1+ and 2.2+ groups in both species, (see Fig. 5). In every case the sea trout growth in fresh water was superior to that of the salmon, but at the end of the first year of sea life and at capture the salmon were superior in length to the sea trout. Similar conditions have been found in other rivers (see Went, 1941. *Proc. Roy. Irish Acad.* 47. B No. 6. p. 174 and Went and Barker, 1943. "*Sci. Proc. R. Dublin Soc.* 23 (N.S.) No. 9. p. 97).

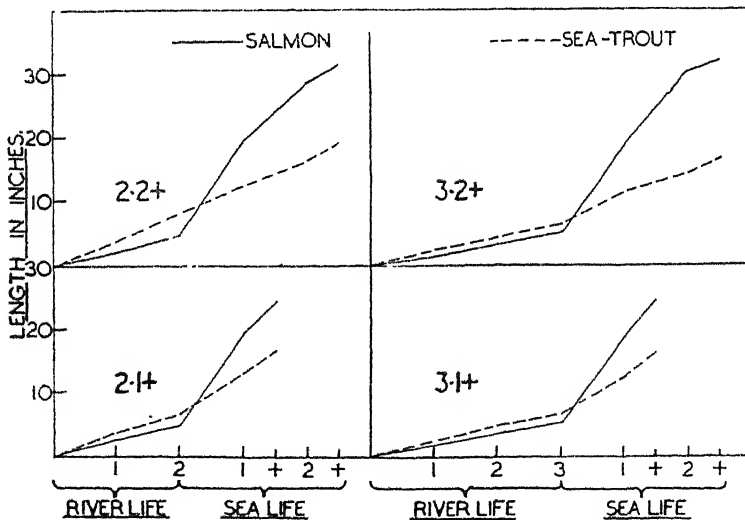


Fig. 5. Comparison of growth in salmon and sea trout in certain categories (See above).

SUMMARY.

1. Material consisting of 747 sets of scales of salmon and 217 sets of scales of sea trout was collected during the years 1941-'44 from the River Inny (Fig. 1).
2. *Salmon.* Two-year smolts accounted for 80.6% of the total and three-year smolts for 16.3% (Table 3). The bulk of the fish were derived from type B smolts

- (Table 4) The grilse formed 85.7 % of the total catch (Table 5) and the proportions of previous spawners (3.1%) and spring fish (4.2%) were small (Tables 5 and 7). A previous spawner which had spawned three times previously was noted (Plate 18). From the table showing divided migration and return (Table 8) it was shown that two-year classes contributed to over 96% of the stock. About $\frac{3}{4}$ of the fish had lengths between 21.95 and 26.95 inches (Table 9 and Fig. 2). Average, maximum and minimum lengths of the various age groups were given in Tables 10 and 12. The proportion of fish having eroded scales and the mean degree of erosion increased as the season progressed. For the end of every year of life the lengths of each maiden or unspawned fish were calculated and the results are given (Table 11-12, 14-15). The fastest growing parr migrated first. (Fig 3.) Grilse were, on the average, longer at the end of the first year in the sea than any other age group (Table 11).
3. *Sea trout.* Two-year smolts formed over $\frac{3}{4}$ of the total (Table 16) two smolt types being observed (Table 17). The fish were divided into the appropriate age groups (Tables 18 and 19). The question of divided migration and return (Table 20) was discussed. The average sizes of the different categories were given in Table 21. The oldest fish were in their seventh year. As in the salmon the growth of each maiden fish was calculated, and the results are given in Tables 22-24. Growth in fresh water was illustrated graphically (Fig 4) when it was shown that the fastest growing parr migrated first.
 4. A comparison of the growth in certain selected age categories of salmon and sea trout was made. (Fig. 5).

APPENDIX

Tables 3 to 15 relate to salmon and Tables 16 to 24 relate to sea trout

Table 1. Chemical analysis of waters of the River Inny (results expressed as parts per 100,000)

| | | | | | |
|-----------------------------|--------|---|----------------|------------------------------|-------|
| Dissolved solids (Volatile) | 1.6 | Nitrates | 0.066 | Calcium Oxide (CaO) | 0.7 |
| (Fixed) | 5.2 | Chlorine | 2.1 | Magnesia (MgO) | 0.1 |
| Saline Ammonia | 0.0015 | Silica (SiO ₂) | 0.8 | Sodium (Na ₂ O) | 2.12 |
| Albuminoid " | 0.019 | Phosphorus (P ₂ O ₅) | Nil | Potassium (K ₂ O) | trace |
| Nitrites | 0.01 | Iron (Fe ₂ O ₃) | less than 0.01 | | |

Table 2. Monthly samples of sets of scales.

| Month | Salmon | | Sea Trout | |
|--------|--------|-------|-----------|-------|
| | No. | % | No. | % |
| April | 3 | 0.4 | 56 | 26.0 |
| May | 41 | 5.5 | 97 | 45.9 |
| June | 102 | 13.7 | 29 | 13.7 |
| July | 510 | 68.3 | 23 | 10.9 |
| August | 63 | 8.4 | 2 | 1.0 |
| Sept. | 28 | 3.7 | 4 | 1.9 |
| Total | 747 | 100.0 | 211* | 100.0 |

* No data for the capture of six fish.

Table 3. Distribution of the different smolt classes in the various age groups

| Smolt Age | Age group (in winters) | | | | Total for Maiden fish only |
|-----------|------------------------|-------|-------|------------|----------------------------|
| | 1+ | 2 | 2+ | With S.Ms. | |
| 1 | 1.5 | 6.2 | 5.8 | 4.4 | 2.1 |
| 2 | 80.1 | 84.4 | 86.5 | 91.2 | 80.6 |
| 3 | 17.3 | 9.4 | 7.7 | 4.4 | 16.3 |
| 4 | 1.1 | - | - | - | 1.0 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

Table 4. Distribution of the different smolt types in the various smolt classes.

| Smolt Age | Smolt type | | | | Total | |
|-----------|------------|------|-----|------|-------|-------|
| | A | | B | | | |
| | No. | % | No. | % | No. | % |
| 1 | - | - | 15 | 2.1 | 15 | 2.1 |
| 2 | 56 | 7.7 | 528 | 72.9 | 584 | 80.6 |
| 3 | 35 | 4.8 | 83 | 11.5 | 118 | 16.3 |
| 4 | 4 | 0.6 | 3 | 0.4 | 7 | 1.0 |
| Total | 95 | 13.1 | 629 | 86.9 | 724 | 100.0 |

Table 5. Percentage of each age group in the catch of each month.

| Month | Age group (in winters) | | | | Total |
|--------|------------------------|-------|------|------------|-------|
| | 1+ | 2 | 2+ | With S.Ms. | |
| April | - | 100.0 | - | - | 100.0 |
| May | 7.3 | 39.1 | 53.6 | - | 100.0 |
| June | 72.5 | 10.8 | 15.7 | 1.0 | 100.0 |
| July | 94.3 | 0.2 | 2.0 | 3.5 | 100.0 |
| August | 90.4 | - | 4.8 | 4.8 | 100.0 |
| Sept. | 89.2 | 3.6 | 3.6 | 3.6 | 100.0 |
| Total | 85.7 | 4.2 | 7.0 | 3.1 | 100.0 |

Table 6. Percentage of the total catch of each age group in each month.

| Month | Age group | | | | Total |
|--------|-----------|-------|-------|------------|-------|
| | 1+ | 2 | 2+ | With S.Ms. | |
| April | - | 9.4 | - | - | 0.4 |
| May | 0.5 | 50.0 | 42.3 | - | 5.5 |
| June | 11.6 | 34.4 | 30.8 | 4.0 | 13.7 |
| July | 75.1 | 3.1 | 19.2 | 78.0 | 68.3 |
| August | 8.9 | - | 5.8 | 14.0 | 8.4 |
| Sept. | 3.9 | 3.1* | 1.9 | 4.0 | 3.7 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

* "dropper"

Table 7. Percentage of spring and summer fish in each month of the season.

| | April | May | June | July | Aug. | Sept. | Total |
|-------------|-------|------|------|------|-------|-------|-------|
| Spring Fish | 100.0 | 39.1 | 10.8 | 0.2 | - | 3.6* | 4.2 |
| Summer Fish | - | 60.9 | 89.2 | 99.8 | 100.0 | 96.4 | 95.8 |

* One fish only a "dropper"

Table 8. Showing divided migration and return as percentages of the total catch for one year.

| Returned in 1941 as | Hatched in the year | | | | | Total |
|--------------------------------|---------------------|------|------|------|------|-------|
| | 1935 | 1936 | 1937 | 1938 | 1939 | |
| Grilse (1+ winters) | - | 0.9 | 14.9 | 68.6 | 1.3 | 55.7 |
| Small spring fish (2 winters) | - | 0.4 | 3.5 | 0.3 | - | 4.2 |
| Small summer fish (2+ winters) | - | 0.5 | 6.1 | 0.4 | - | 7.0 |
| Previous spawners (with S.Ms.) | 0.1 | 0.3 | 2.6 | 0.1 | - | 3.1 |
| Total | 0.1 | 2.1 | 27.1 | 69.4 | 1.3 | 100.0 |

Table 9. The estimated size distribution of salmon as percentages of the year's catch.

| Class Interval* | Age group (in winters) | | | | Total |
|-----------------|------------------------|-----|-----|-----------|-------|
| | 1+ | 2 | 2+ | With S.M. | |
| 18 | 0.1 | - | - | - | 0.1 |
| 19 | 0.4 | - | - | - | 0.4 |
| 20 | 2.7 | - | - | - | 2.7 |
| 21 | 4.6 | - | - | - | 4.6 |
| 22 | 8.6 | - | - | - | 8.6 |
| 23 | 13.0 | - | - | - | 13.0 |
| 24 | 20.1 | - | - | 0.1 | 20.2 |
| 25 | 18.0 | - | - | - | 18.0 |
| 26 | 12.2 | 0.3 | - | 0.5 | 13.0 |
| 27 | 4.4 | - | 0.1 | 0.2 | 4.7 |
| 28 | 0.9 | 0.5 | 0.3 | 0.5 | 2.2 |
| 29 | 0.4 | 0.5 | 0.8 | 0.8 | 2.5 |
| 30 | 0.3 | 1.7 | 1.5 | 0.2 | 3.7 |
| 31 | - | 0.7 | 1.6 | 0.4 | 2.7 |
| 32 | - | 0.3 | 1.1 | 0.2 | 1.6 |
| 33 | - | 0.1 | 1.1 | - | 1.2 |
| 34 | - | - | 0.4 | 0.1 | 0.5 |
| 35 | - | 0.1 | - | 0.1 | 0.2 |
| 36 | - | - | 0.1 | - | 0.1 |
| Total | 85.7 | 4.2 | 7.0 | 3.1 | 100.0 |

* 18 etc. includes all fish between 17.95 inches and 18.95 inches etc.

Table 10. Average length in inches of the grilse in each month of the season.

| Month | No. | Mean Length | Estimated weight * |
|-----------|-----|-------------|--------------------|
| May | 3 | 24.3 | 5.7 |
| June | 63 | 23.2 | 5.0 |
| July | 508 | 24.7 | 6.0 |
| August | 41 | 25.0 | 6.2 |
| September | 25 | 26.3 | 7.3 |
| Total | 640 | 24.5 | 5.9 |

* On basis of K = 1.1 (see text)

APPENDIX—*continued.*

Table 20. Divided migration and return showing the years in which the fish were hatched.

| Returned in 1944 as fish in their | Hatched in | | | | | Total |
|-----------------------------------|------------|------|------|------|------|-------|
| | 1938 | 1939 | 1940 | 1941 | 1942 | |
| 1st Post-migration year | — | — | — | 1 | 2 | 3 |
| 2nd ditto. | — | 3 | 19 | 112 | 9 | 143 |
| 3rd ditto. | — | 1 | 4 | 1 | — | 6 |
| 4th ditto. | — | 1 | — | — | — | 1 |
| Previous spawners | 9 | 18 | 31 | 6 | — | 64 |
| Total | 9 | 23 | 54 | 120 | 11 | 217 |

Table 21. Mean lengths in inches in the different age categories.

| Age Category | No. | Mean length in ins. | Age Category | No. | Mean length in ins. |
|--------------|------|---------------------|--------------|-----|---------------------|
| 2.+S.M.+ | 4 | 15.7 | 2.1+2 S.M.+ | 12 | 20.5 |
| 2.+2 S.M.+ | 4 | 18.5 | 2.1+3 S.M.+ | 5 | 21.2 |
| 2.+3 S.M.+ | 1 | 18.3 | 3.1+ | 19 | 16.5 |
| 3.+ | 2 | 12.8 | 3.1+S.M.+ | 3 | 17.8 |
| 3.+S.M.+ | 3 | 15.7 | 3.1+2 S.M.+ | 3 | 20.2 |
| 4.+ | 1 | 14.5 | 4.1+ | 3 | 16.9 |
| 4.+S.M.+ | 1 | 16.9 | 4.1+S.M.+ | 1 | 17.0 |
| 1.1+ | 9 | 15.5 | 1.2+ | 1 | 15.9 |
| 1.1+S.M.+ | 2 | 17.2 | 2.2+ | 4 | 19.0 |
| 1.1+2 S.M.+ | 1 | 20.1 | 2.2+S.M.+ | 1 | 20.3 |
| 2.1+ | 111* | 16.4 | 3.2+ | 1 | 16.6 |
| 2.1+S.M.+ | 23 | 17.6 | 2.3+ | 1 | 17.5 |

* One not measured.

Table 22. Growth (in inches) in fresh water in the different smolt classes.

| Smolt Age | No. | Length at end of | | | | Mean Smolt Length |
|-----------|-----|------------------|----------|----------|----------|-------------------|
| | | 1st Year | 2nd Year | 3rd Year | 4th Year | |
| 1 | 10 | 4.2 | — | — | — | 6.4 |
| 2 | 113 | 3.3 | 6.6 | — | — | 7.5 |
| 3 | 22 | 2.5 | 4.9 | 6.9 | — | 7.6 |
| 4 | 4 | 2.1 | 4.6 | 7.1 | 8.9 | 9.6 |

Table 23. Estimated growth in inches in the various age categories.

| Age Category | No. | Years in River | | | | Mean Smolt Length | Years in Sea | | Mean Length at Capture |
|--------------|------|----------------|-----|-----|------|-------------------|--------------|------|------------------------|
| | | 1 | 2 | 3 | 4 | | 1 | 2 | |
| 3.+ | 2 | 2.5 | 5.2 | 8.6 | — | 9.8 | — | — | 12.8 |
| 4.+ | 1 | 2.2 | 5.7 | 7.9 | 10.8 | 11.8 | — | — | 14.5 |
| 1.1+ | 9 | 4.2 | — | — | — | 6.5 | 11.9 | — | 15.5 |
| 2.1+ | 109* | 3.4 | 6.5 | — | — | 7.5 | 12.6 | — | 16.4 |
| 3.1+ | 19 | 2.5 | 4.9 | 6.8 | — | 7.4 | 12.3 | — | 16.5 |
| 4.1+ | 3 | 2.1 | 4.4 | 6.8 | 8.3 | 8.8 | 13.4 | — | 16.9 |
| 1.2 | 1 | 4.0 | — | — | — | 5.8 | 9.3 | 12.7 | 15.9 |
| 2.2 | 4 | 3.8 | 8.1 | — | — | 8.8 | 12.5 | 16.1 | 19.0 |
| 3.2 | 1 | 2.3 | 4.4 | 6.7 | — | 8.0 | 11.6 | 14.3 | 16.6 |

* Three fish not measured.

Table 24. Estimated growth in inches in various age groups.

| Age group. | No. | Mean smolt length | Years in Sea | | Mean length at capture |
|------------|-----|-------------------|--------------|------|------------------------|
| | | | 1 | 2 | |
| + | 3 | 10.5* | — | — | 13.4* |
| 1+ | 140 | 7.4 | 12.5 | — | 16.3 |
| 2+ | 6 | 8.2 | 11.8 | 15.2 | 18.1 |

* Only older smolt classes included (See text).



PLATE —Photograph of a scale of a salmon (35 inches long) which had spawned on three previous occasions captured on 19th July, 1941. Magnification x 25

No. 30.

THE DIAZO REACTION IN THE TETRAZOLE RING.

By

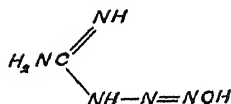
J. REILLY, J. P. TEEGAN AND M. F. CAREY.

[Read MARCH 25, 1948 Published separately SEPTEMBER 29, 1948]

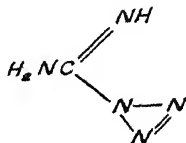
THE diazotisation of primary amines with the formation of diazonium salts is a characteristic reaction of practically all aromatic and many heterocyclic ring systems (1, 2, 3). For diazotisation, unsaturation *and* carbon attached thus.—

$\equiv\text{CNH}_2$ appear to be essential, *i.e.* unsaturated carbon attached to the amino group with a second centre of unsaturation in the complex. Even here some heterocyclic ring systems are exceptional. In the case of open chain compounds, aminoguanidine is in a unique position. In some ways it appears to be in line with aromatic primary amines, but the conditions of the reaction are quite distinct, with tetrazole ring formation in some cases.

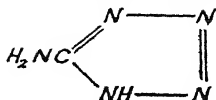
The normal method of diazotisation with sodium nitrite in the presence of free mineral acid yields, with aminoguanidine salts, salts of a compound considered by Thiele (4) to be "diazoguanidine"



but which subsequently was shown by Hantzsch and Vagt (5) to be guanyl azide.—

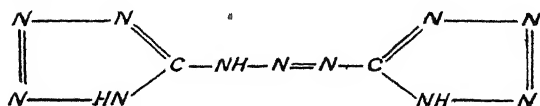


This azide on treatment with a weak base isomerises to 5-aminotetrazole:—



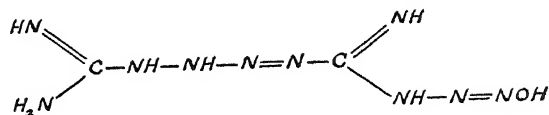
which on diazotisation gives a diazonium salt that couples with components such as α - and β -naphthylamine and β -naphthol, as was noted by Thiele (4).

Replacement of mineral acid by glacial acetic acid leads to the formation of 1-3 ditetrazolyltriazene.—

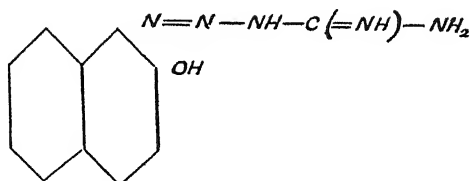


which was shown by Hofmann and Hock (6) to give coloured solutions with intermediates. In the thermal decomposition of 1-3 ditetrazolyltriazene, these authors obtained nitrogen with a small amount of cyanogen. The latter substance is probably due to working with a product contaminated with the open chain diazo-hydroxide.

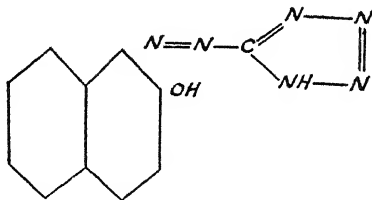
Hofmann and Roth (7, 8) have claimed that if aminoguanidine nitrate (or sulphate) in aqueous solution is treated with sodium nitrite under special conditions there results a true aliphatic diazonium hydroxide:—



This hydroxide is also claimed to couple slowly with α - and β -naphthylamine and m-phenylenediamine to give coloured (azo) solutions. The coloured compound from β -naphthol has been prepared and examined by Norris-Shreve, Carter and Willis (9) who gave the structure:—

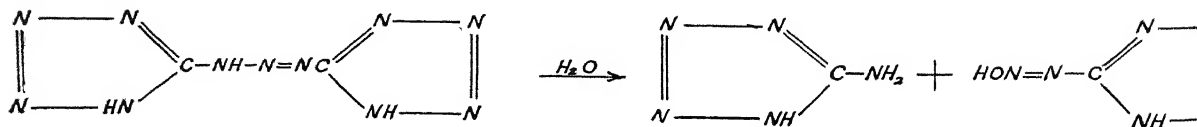


In the present work a comparison has been made between the β -naphthol derivative obtained from 1-3-ditetrazolyltriazene and that from Hofmann and Roth's diazonium hydroxide. Experimental evidence shows that they are the same compound, namely 1-2-3-4-tetrazole-5-azo- β -naphthol:—

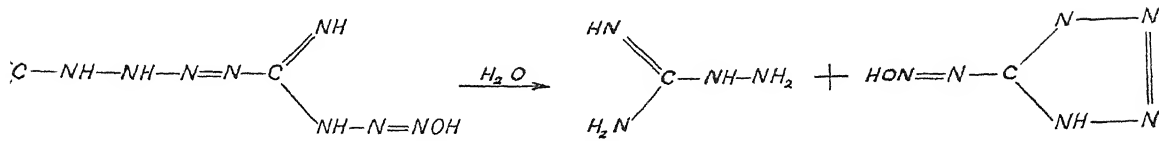


The same compound was also obtained by diazotising 5-aminotetrazole and coupling this with β -naphthol.

In the case of the 1-3-ditetrazolyltriazene, during the coupling reaction, which is carried out in aqueous solution at 80°C, it has been shown (see Experimental Section) that splitting occurs at the $-\text{NH}-\text{N}=\text{N}-$ link with the formation of 5-aminotetrazole and tetrazole-5-diazonium hydroxide.



The diazonium complex was confirmed by the formation of an azo derivative. When no intermediate is present the diazonium hydroxide loses nitrogen, and forms 5-hydroxytetrazole. In the case of the straight-chain diazonium hydroxide it has been found that a secondary reaction is involved. Splitting occurs, as before, at the $\text{-NH}-\text{N}=\text{N}-$ link, followed by ring closure and the formation of tetrazole 5-diazonium hydroxide —



The tetrazole 5-diazonium hydroxide then couples with the β -naphthol in the normal manner. The azo derivative is therefore that of a heterocyclic-aromatic grouping and not an aliphatic-aromatic complex.

Preliminary experiments with the straight-chain diazonium hydroxide in inert solvents gave no evidence of intramolecular isomerism. This point however, is being further investigated.

The structure of the foregoing two β -naphthol derivatives was shown by reductive splitting with the formation of α -amino- β -naphthol and 5-amino-tetrazole.

EXPERIMENTAL.

Preparation of β -naphthol derivative from 1-3-ditetrazolyltriazene.

4 g. (0.027 mol.) β -naphthol were added to 300 ml. of water and the whole heated to 80°C , which temperature was maintained throughout the entire experiment. 6.25 g. (0.027 mole) of 1-3-ditetrazolyltriazene (*disodium salt*) were now added slowly over a period of fifteen minutes with continual stirring. During the addition, a deep red colour developed accompanied by a small evolution of nitrogen. On cooling, unreacted β -naphthol was filtered off. 5 ml. of concentrated hydrochloric acid solution were added to precipitate the orange-coloured β -naphthol derivative. This was purified by recrystallisation from toluene (cf. Norris-Shreve *et. al.* (9)).

In its general properties, this compound closely resembled that prepared by Norris-Shreve *et. al.* from Hofmann and Roth's diazonium hydroxide. It was almost completely insoluble in cold water, slightly less so in hot, the solution having an orange colour. It was insoluble in benzene, ether, chloroform. It dissolved readily in solutions of alkalis, even in such weak bases as pyridine, giving a red solution. M.P. (when recrystallised from toluene) 165°C . Found, C, 55.0; H, 3.0; N, 36.3; $\text{C}_{11}\text{H}_8\text{ON}_6$ requires: C, 55.0; H, 3.3; N, 35.0.

A sample of the β -naphthol derivative as obtained from the diazonium hydroxide by Norris-Shreve, Carter, and Willis (8) was now prepared. The properties of this derivative coincided closely with those already given for that prepared from 1-3 ditetrazolyltriazene, and the mixed melting point was 165°C . Found, N, 35.6; $\text{C}_{11}\text{H}_8\text{ON}_6$ requires N, 35.0.

The presence of one azo link in the molecule of the β -naphthol complex was determined volumetrically with standard titanous chloride solution.

Decomposition of 1-3-ditetrazolyltriazene (disodium salt) on heating to 80°C. in aqueous solution.

0.182 g. of 1-3-ditetrazolyltriazene (*disodium salt*) was heated to 80°C in 50 ml. water and the rate of evolution of nitrogen, with time, studied by collecting the gas in a water-cooled gas burette. As shown in Table I two atoms of nitrogen were lost per molecule of triazene, and the reaction followed a monomolecular course. The nitrogen evolved was free from cyanogen.

TABLE I.

| Time (t) | Vol. $\left\{ \begin{smallmatrix} 759 \\ 20^{\circ} \end{smallmatrix} \right.$ | Vol. N.T.P. | K_1 (mono). 10^{-1} |
|----------|--|-------------|-------------------------|
| 1 min. | 1.9 c.c. | 1.76 c.c. | 1.040 |
| 2 " " | 3.5 " " | 3.37 " " | 1.042 |
| 3 " " | 5.2 " " | 4.81 " " | 1.044 |
| 4 " " | 6.6 " " | 6.12 " " | 1.047 |
| 5 " " | 7.8 " " | 7.30 " " | 1.048 |
| 6 " " | 8.9 " " | 8.33 " " | 1.048 |
| 7 " " | 10.0 " " | 9.30 " " | 1.048 |
| 9 " " | 11.8 " " | 10.94 " " | 1.050 |
| 11 " " | 13.5 " " | 12.54 " " | 1.055 |
| 3 hrs. " | 19.2 " " | 17.90 " " | -- |

This final figure of 17.9 c.c. corresponds with an evolution of one molecule of nitrogen from 1 molecule of 1-3-ditetrazolyltriazene (*disodium salt*) $C_2N_{11}HNa_2$ (0.182 mol. theoretically yields 18.1 c.c. at N.T.P.).

The resulting colourless solution was shaken with ether. The ether layer on evaporation yielded colourless crystals, which were identified as 5-hydroxy-tetrazole (M.P. 254°C). The aqueous layer, on evaporation, gave a residue which was recrystallised from alcohol, giving a crystalline compound M.P. 203°C. It was identical with 5-amino-tetrazole, and a mixed melting point showed no alteration.

Reductive splitting of β -Naphthol complex.

This was effected, first, by using sodium hyposulphite. 0.3 g. of the complex was suspended in 100 ml. water at 100°C and 2.5 g. of powdered sodium hyposulphite were slowly added. Heating was continued until the orange colour due to the complex had disappeared. 5 ml. of concentrated hydrochloric acid solution were added which gave a precipitate. This was filtered off and shown to be α -amino- β -naphthol hydrochloride.

To a portion of the filtrate a solution of copper sulphate containing some sodium acetate was added, which gave a heavy green precipitate of the copper salt of 5-aminotetrazole (4). The remainder of the filtrate from the α -amino- β -naphthol was carefully evaporated, and crystals of 5-aminotetrazole hydrochloride separated. Reduction with tin and hydrochloric acid solution yielded the same products.

Preparation of β -Naphthol complex from diazotised 5-aminotetrazole and β -naphthol.

5-Aminotetrazole was prepared by diazotising aminoguanidine nitrate in the presence of free mineral acid, isolating the guanyl azide and refluxing this with sodium acetate solution. This base in solution was treated with dinitrogen trioxide in chloroform at 0°C with constant stirring until it reacted to starch iodide paper. The lower chloroform layer was separated. The upper layer containing the diazonium derivative was treated with urea to destroy excess nitrous acid. A few drops of dilute hydrochloric acid solution were added. To this was added some β -naphthol dissolved in very dilute sodium hydroxide solution. An orange coloured precipitate developed. The compound, recrystallised from toluene, had M.P.=164°C. Found N, 34.5. $C_{11}H_8ON_6$ requires N, 35.0.

1-3-ditetrazolyltriazene and Hofmann and Roth's diazonium hydroxide coupled at 80°C in aqueous solution with β -naphthol gave the same azo derivative.

An acknowledgment is made to the Industrial Research Council of Ireland for grants to two of the authors, J.P.T. and M.F.C.

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